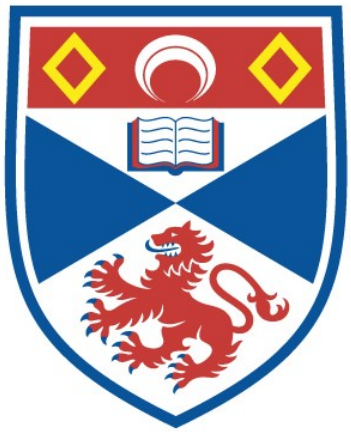
RESPIRATION TIMING AND UNDERWATER ACTIVITY IN KILLER WHALES (ORCINUS ORCA)

Marjoleine Merel Hansje Roos

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Marjoleine Merel Hansje Roos

This thesis is submitted in partial fulfilment for the degree of MPhil
at the
University of St Andrews

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A north Atlantic killer whale breaking the surface to breathe (photo by M.M.H. Roos, 2013)

Declarations

Candidate's declarations:

I, Marjoleine M.H. Roos, hereby certify that this thesis, which is approximately 40,000 words in length, has been written by me, and that it is the record of work carried out by me or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in June 2012 and as a candidate for the degree of Master of Philosophy in June 2012; the higher study for which this is a record was carried out in the University of St Andrews between 2012 and 2015.

Date 26th January 2015

Marjoleine M. H. Roos

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I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of M.Phil. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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Marjoleine M.H. Roos

Dr. Patrick J.O. Miller

Collaboration statement

Data collection fieldwork was concluded as part of the 3S project, a collaboration to study the effects of sonar on cetaceans, with partner SMRU, WHOI, TNO, and FFI, Norway. Statistical advice was provided by Dr. Mick Wu. Advice on O_2 uptake curve equations was provided by Dr. Dave Thompson.

Abstract

Accurate estimates of energetic requirements of top predators *in-situ* are essential to improve sustainable marine ecosystems' management. Yet, obtaining direct energetic measurements of free-ranging cetaceans is unfeasible. Breathing rate has been used as indicator of cetacean metabolic rates, though rate alone does not account for breath-by-breath variation in gas exchange. This study's aim was to investigate 1) the strength of correlations between respiration rates and underwater activity levels; and 2) the potential influence of including respiratory timing (besides rate) and oxygen uptake dynamics on *in-situ* cetacean energetic studies. Kinematic data from 12 adult wild Norwegian herring-feeding killer whales (*Orcinus orca*) were recorded with high-resolution tags (DTAGs) to reveal individual breathing events. Three-axis accelerometer and flow noise data were used to derive stroking rate and speed as underwater activity level metrics. An oxygen exchange model, including an oxygen uptake curve as key feature, and oxygen consumption from swimming speed or stroke number, was established to estimate oxygen extraction dynamically per individual breath, based upon modelled oxygen store at the time of each breath. Correlations between predicted oxygen uptake and activity level over 15 min periods were relatively weak when using constant uptake per breath (for both speed and stroking $r^2 < 0.1$). Including fluctuating oxygen uptake per breath significantly improved the correlation between modelled oxygen uptake and activity (for both speed and stroking $r^2 > 0.9$). Model outcomes found that cost of transport kept decreasing when assuming fixed oxygen uptake, whereas applying fluctuating uptake revealed a clear minimum cost of transport speed of 2.0-2.5 $m \cdot s^{-1}$. With on-going development and effort concerning bio-logging, together with gain of accurate information on energetic costs relating to kinematics, the proposed model could become a useful tool to improve our knowledge of free-ranging cetaceans' energetic requirements in relation to more advanced sustainable marine ecosystem management.

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1. General introduction

Metabolic rate (MR) can be defined as 'the rate at which animals use energy' (Schmidt-Nielsen, 1997). Kleiber (1975) defined basal metabolic rate (BMR) as the rate of energy use necessary to survive for resting, mature (non-pregnant) animals, during the post-absorptive state while being thermal-neutral. Additional energy is required for growth, reproduction, healing, waste, or locomotion (Costa, 2009). Like all other animals, marine mammals have to make trade-offs how to allocate their energy, in such a way which will maximize their long-term reproductive success.

1.1. Measuring metabolic rates of marine mammals

Definitions of acronyms and units	
ADL	Aerobic dive limit (min)
BMR	Basal metabolic rate (L $O_2 \cdot s^{-1}$)
CO_2	Carbon dioxide (L)
COT	Cost of transport (L $O_2 \cdot kg^{-1} \cdot m^{-1}$)
E_{O2}	Oxygen extraction (% or L)
k	Slope of MR_L vs. activity (speed^3 or stroking rate)
MR	Metabolic rate (L $O_2 \cdot s^{-1}$)
O_2	Oxygen (L)
SPL	Sound pressure level (dB re 1 µPa)
TBO	Total body oxygen store (L)
V_{O2}	Oxygen consumption rate (L $O_2 \cdot s^{-1}$)
V_T	Tidal lung volume (L)

For many decades scientists have put effort in studying the metabolism of marine mammals to quantify their impact on ecosystems and create a better understanding of their adaptations to the aquatic environment. MR has been measured in marine mammals using a variety of techniques (Table 1.1).

Many studies on marine mammal metabolism to date have emphasized locomotion and activity levels (Castellini *et al.*, 1992; Williams *et al.*, 1993; Hind and Gurney, 1997; Williams *et al.*, 2004a; Wilson *et al.*, 2006; Fahlman *et al.*, 2008a; Fahlman *et al.*, 2013; Gerlinsky *et al.*, 2013). Marine mammals face hydrodynamic constraints during locomotion, such as drag, which is dependent upon body size, shape and travel speed (Williams and Worthy, 2002). Yet, they do not always carry their own weight like terrestrial animals, because at times they are close to neutrally buoyant (Miller *et al.*, 2004; Aoki *et al.*, 2011).

Table 1.1. Standard methods used for estimating metabolic rates in marine mammals

Method	Description	References and species
Respiration rate	Number of breaths per time unit multiplied by V_T	Cetaceans: Sumich (1983) (grey whale); Dolphin (1987) (humpback whale); Blix and Folkow (1995) (minke whale); Rodríguez de la Gala-Hernández *et al.* (2008) (grey whale); Williams and Noren (2009) (killer whale); Christiansen *et al.* (2014) (minke whale)
Oxygen respirometry	Estimation of O_2 consumption through direct measurement of O_2 (and CO_2) in exhaled gases	Pinnipeds: Feldkamp (1987) (California sea lion); Sparling and Fedak (2004) (grey seal); Fahlman *et al.* (2008a) (Steller sea lion); Dassis *et al.* (2012) (Southern sea lion); Dalton *et al.* (2014) (northern fur seal) Cetaceans: Kasting *et al.* (1989) (killer whale, beluga) Williams *et al.* (1993) (bottlenose dolphin); Kriete (1995) (killer whale); Worthy *et al.* (2013) (killer whale)
Doubly labelled water	Replaces hydrogen and O_2 in water with an isotope to measure water and CO_2 flow in the body over time	Pinnipeds: Costa and Gales (2003) (Australian sea lion); Acquarone *et al.* (2006) (walrus); Sparling *et al.* (2008); Dalton *et al.* (2014) (northern fur seal)
Heart rate	Heart rate is related to the rate of O_2 flow to body tissues	Pinnipeds: Young *et al.* (2011) (Steller sea lion) Cetaceans: Reed *et al.* (2000) (harbour porpoise); Noren *et al.* (2004) (bottlenose dolphin)

Marine mammals have evolved to live underwater and have the ability to decrease their metabolism to save oxygen (O_2) during prolonged dives as one of the key features of the dive response (Hurley and Costa, 2001; Hastie *et al.*, 2007). Still, they are forced to come to the surface to recover O_2 they consume during each dive. This study is focused on aerobic

metabolism for which O_2 is needed to convert fats, carbohydrates and proteins into energy in the form of ATP, simultaneously producing carbon dioxide (CO_2) and water. Since the amount of heat formed by the usage per liter of O_2 is approximately equal during oxidation of either fat, carbohydrate or protein, O_2 is a concrete measure of MR (Schmidt-Nielsen, 1997). O_2 respirometry is a frequently practiced technique for obtaining data required to calculate the rate of O_2 consumption (V_{O2}) and refers to the direct measurement of O_2 (and CO_2) in exhaled gasses (Fedak *et al.*, 1981).

There are two variants of respirometry. Open-flow respirometry is most commonly used for marine mammals in captivity (Williams *et al.*, 1993; Otani *et al.*, 2001; Sparling and Fedak, 2004; Fahlman *et al.*, 2008a; Rosen and Trites, 2013; Worthy *et al.*, 2013). In this approach the animal breathes within a metabolic chamber through which air is drawn constantly to replenish the O_2 that is consumed, while CO_2 and water vapour exhaled by the animal is discarded (Iverson *et al.*, 2010). Gas samples are taken from the extracted air at time intervals to measure V_{O2} and CO_2 production over a period of time (Iverson *et al.*, 2010). To collect gasses from large cetaceans the open-flow set-up is rather undesirable due to their required size, but it has been used in some studies of large cetaceans (Rosen and Trites, 2013; Worthy *et al.*, 2013). For these animals O_2 extraction (E_{O2}) and CO_2 production has also been measured in another form of respirometry by tightly placing a large balloon or bag over the blowhole for collecting exhaled air per breath from which samples will be taken (Sumich, 1983; Kasting *et al.*, 1989; Kriete, 1995; Sumich, 2001).

A debate has existed whether marine mammals have elevated metabolic rates compared to terrestrial mammals (Kasting *et al.*, 1989; Innes and Lavigne, 1991; Kasting, 1991; Gallivan, 1992; Speakman *et al.*, 1993; Gallivan, 1995). Uncertainty about representative metabolic rate values arises from the fact that valid data is complicated to obtain. Though direct measurements on captive cetaceans are possible, most of these are focussing on smaller species, including the harbour porpoise (Reed *et al.*, 2000; Boutilier *et al.*, 2001), Pacific white-sided dolphin (Rechsteiner *et al.*, 2013), and bottlenose dolphin (Williams *et al.*, 1993; Williams *et al.*, 1999; Yazdi *et al.*, 1999; Noren *et al.*, 2004). Two of the few studies on metabolic rates of larger cetaceans in captivity focussed on one grey whale calf (Sumich, 2001) and killer whales (Kriete, 1995). Apart from these few studies, there is a general lack of direct metabolic rate measurements of large cetaceans.

1.2. Metabolic rates of free-ranging cetaceans

Accurate estimates of energetic requirements of cetaceans at sea are essential to improve sustainable management of marine ecosystems. Dynamics of cetacean populations alone or in combination with other environmental factors can have a significant impact on marine ecosystems (Jefferson *et al.*, 1991; Estes *et al.*, 1998; Plagányi and Butterworth, 2009). If meaningful information on energetic requirements per individual were available, cetacean energy uptake and subsequent influence on the marine ecosystem could be modelled providing long-term forecasts, including concerning fish stocks. Also, immediate and long-term competition between cetacean populations, commercial fisheries and other marine mammal species preying upon similar prey species can be analysed to examine whether this competition potentially limits growth, reproduction and survival of any of the predator species populations.

However, despite the importance of metabolic rates of large cetaceans, direct data collection on metabolic rates from these animals remains a challenge, if not unfeasible for many species. Certain methods that require captivity can only be carried out for taxa that can be successfully held in captivity. Free-ranging cetaceans often have a large habitat range and spend most of their lifetime under water, while feeding, mating, and during other activities. Due to these challenges data collection on these inaccessible creatures in general is limited to behavioural surface observations or the use of telemetry (see Section 1.4 and 1.5, respectively).

1.3. Respiration rate as metric for metabolic rate

For an animal with discrete breaths, MR (the rate of V_{O2}) can be calculated using the following equations:

$$E_{O2} = I_{O2} - X_{O2}, \quad (1)$$

Where, E_{O2} is the proportion of O_2 extracted from the inhaled air, I_{O2} is the available O_2 in the inhaled air, and X_{O2} is the proportion of O_2 in the exhaled air. The amount of O_2 exchanged per breath is:

$$T_{O2} = V_T * E_{O2}, \quad (2)$$

Where, T_{O2} is amount of O_2 exchanged ($L \cdot breath^{-1}$) and V_T is tidal lung volume ($L \cdot breath^{-1}$), the amount of air exchanged during a single respiration (Wartzok, 2002). By measuring the number of breaths per unit time, V_{O2} per unit time can be calculated.

During early captive studies on marine mammal metabolism, using respirometry, it was observed that breathing rate itself increased with an increase in V_{O2} per minute (Scholander and Irving, 1941; Hampton *et al.*, 1971; Hampton and Whittow, 1976). Historically, scientists studied metabolism of captive marine mammals using breathing rate as a measurement (Irving *et al.*, 1941; Scholander and Irving, 1941; Spencer *et al.*, 1967; Olsen *et al.*, 1969). This method has been explored by other scientists, which found supporting evidence that breathing rate is a reliable metric for MR (Yazdi *et al.*, 1999; Sumich, 2001).

As respiration 'blows' of cetaceans can be seen from a distance, breathing rate has been a vital tool for estimating MR of cetaceans *in-situ* (field MR) ever since, including for some key studies on free-ranging large whale species (Sumich, 1983; Dolphin, 1987; Blix and Folkow, 1995; Kriete, 1995; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014) (Table 1.1, Appendix 1.2). For these listed studies breathing rate from free-ranging specimens, is multiplied with an average V_T value, which is measured from animals in captivity. Normally, a conversion factor of 20.1 $kJ \cdot LO_2^{-1}$ or 4.83 $kcal \cdot LO_2^{-1}$ is used, and a percentage of 20.95 of O_2 in air, to produce energetic estimations. These were then linked to either a certain behaviour, such as feeding, travelling and resting (Dolphin, 1987; Blix and Folkow, 1995), or speed (Sumich, 1983; Blix and Folkow, 1995; Kriete, 1995; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014).

1.4. Surface observations versus underwater movements

Recent developments in telemetry make it possible to quantify small-scale behaviour over prolonged periods that can be expected to cause direct variation in MR and which cannot be observed from surface observations (Hooker *et al.*, 2007). Activity metrics for animals at sea include stroking rate, speed, and overall dynamic body acceleration (ODBA) (Table 1.2). In

some species correlations between these small-scale metrics and V_{O2} have been found (see references Table 1.2). Therefore, these metrics show potential to serve as key proxies for V_{O2} in free-ranging divers.

Table 1.2. Activity metrics collected through telemetry used to estimate small-scale metabolic rates

Metric	Explanation	References
Speed	Speed directly influences the amount of drag an animal experiences and power requirements should therefore increase as s non-linear function of speed.	Feldkamp (1987); Hind and Gurney (1997); Goldbogen *et al.* (2006); Hassrick *et al.* (2007); Williams and Noren (2009)
Stroking rate	Aquatic animals either use their wings, flippers or flukes to create thrust. Through telemetry data strokes are visual either through oscillation in accelerometers or (concerning larger cetaceans) in the dive profile. Metabolic cost of strokes has been estimated as a constant value (per stroke).	Williams *et al.* (1993); Fish (1998); Williams *et al.* (2004a); Goldbogen *et al.* (2006); Iverson *et al.* (2010)
ODBA	The 3-dimensional raw acceleration minus the constant acceleration downwards caused by earth's gravity produces absolute dynamic acceleration caused by activity of the animal.	Fahlman *et al.* (2008b); Halsey *et al.* (2009); Gleiss *et al.* (2011)

1.5. Collecting telemetry data using animal-attached data loggers

Making quantitative predictions concerning respiration behaviour of a diver involves knowledge of the MR per behavioural state during the dive phase, an aspect which is commonly simplified by using a constant MR (Thompson *et al.*, 1993; Houston, 2011). For all studies listed in Appendix 1.2, except for Blix and Folkow (1995), it was assumed that behaviour observed at the surface was an accurate reflection of overall behaviour while small-scale changes in behaviour or activity were ignored. Yet, surface behaviour may indicate little about the activities of a cetacean underwater. Besides, short-duration behaviours can potentially have a significant impact on MR if they are energetically costly (Gallivan, 1992; Skrovan *et al.*, 1999; Williams *et al.*, 1999; Williams *et al.*, 2004a). For example, lunge feeding in baleen whales can incur a relatively high energetic cost (Goldbogen *et al.*, 2012). Also, to measure speed accurately one needs to know the total distance travelled, which is difficult to

confirm from surface observations alone as it is impossible to measure the dive depth or underwater excursions of a submerged animal. Surface estimates of speed are based upon the minimum possible travel distance between two surfacing points (Sumich, 1983; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014) and could therefore have resulted in an overestimation of the MR per unit distance. Kriete (1995) however, tried to address this concern by adding a dive model to her movement data.

Advancing technology, including archival data-loggers and/or data telemetry devices is crucial to monitoring animal behaviour beneath the water surface or in the open ocean (Hooker *et al.*, 2007). Digital acoustic recording tags or DTAGs (Johnson and Tyack, 2003) provide an important tool to describe marine mammal behaviour *in-situ* due to their ability to record fine-scale movements related to underwater and surfacing activities. Critical features of DTAGs include a 50 Hz sampling rate of sensor data including depth and 3-axis measurement of acceleration and the magnetic field, which can be used to determine the dive profile and orientation (pitch, roll and heading) of the tagged animal (Johnson and Tyack, 2003). Synchronously, audio is recorded at a rate of 96 kHz. Through DTAG deployments, diving behaviour of cetaceans at sea can be recorded directly and their use in the present study created a unique opportunity to undertake fine-scale continuous data collection of consecutive dives, including timing of respiration events (Miller *et al.*, 2010) and fine-scale underwater movements, of free-ranging killer whales. Data on respiration timing would be more difficult to collect from marine mammal species other than cetaceans, otariids, and manatees, which are 'single-breathers' taking a single breath per surfacing (Wartzok, 2002). Whereas cetaceans typically perform a series of successive surfacings after a deeper dive, phocid seals float at the surface while breathing repeatedly without diving underwater (Wartzok, 2002).

1.6. Time scales of data collection

The length of time intervals over which breathing rate are measured can have influence on outcomes; too short intervals can result in an overestimation of breathing rate (Kriete, 1995; Williams and Noren, 2009), though, by using too long intervals small-scale MR changes will be impossible to reveal. Blix and Folkow (1995) did distinguish feeding behaviour using surface observations, yet found no elevated costs during this behaviour. A plausible explanation for

this finding is that small scale changes in behaviour and MR would not have been detected due to their method of averaging breathing rate over randomly chosen 30 minute time intervals. Over that time interval only the dominant behaviour was considered. Using this method it could have been anticipated that no significant variations in MR would be found since a change in V_{O2}, caused for example by a lunge, might only be detectable on a smaller time scale (Gallivan, 1992). Measurements on breathing rate and speed made by Williams and Noren (2009) were averaged over a time interval of at least 15 minutes of observation of the focal whale. Therefore, again, no smaller scale measurements on variation in MR estimates were attempted.

1.7. Killer whales

Killer whales (*Orcinus orca*, Linnaeus 1758) are the largest member in the *Delphinidae* family (order *Cetartiodactyla*, suborder *Odontoceti*) ranging widely across all oceans, yet showing a preference to temperate waters and areas of high productivity (Baird, 2000). The conservation status of the species as a whole is labelled as ”Data deficient” because of uncertainty concerning possible subspecies and their corresponding numbers (IUCN, 2014). However, several well-studied northeast Pacific populations are known to show or have shown rapid declines in numbers and are listed under different acts as threatened, endangered or depleted (Krahn *et al.*, 2002; Saulitis *et al.*, 2005; Fisheries and Oceans Canada, 2008). While for poorly-known populations there is a lack of data to make adequate estimation on population sizes (Jones, 2006).

The most thorough research on killer whales started in the 1970’s in the Northeast Pacific off the coast of British Columbia, focusing on abundance, social structure, diet, behaviour and acoustics (Baird, 2000; Ford *et al.*, 2000). Killer whales are highly social animals forming stable groups referred to as ‘pods’ (Bigg *et al.*, 1990). Their social structures within these pods tend to depend on prey specialization (Baird and Dill, 1996; Ford, 2009). Resident fish-eating populations form pods of ten to tens of individuals formed by matrilines; both males and females remain with their natal group for the rest of their lives (Ford *et al.*, 2000). Group complexity is indicated for instance by alloparental care, by not only females but also adult males (Bisther and Vongraven, 1993; 1995). In contrast, mammal-eating specimens form

smaller groups deprived of matrilines (Baird and Dill, 1996; Baird and Whitehead, 2000).

For these populations it was discovered that they potentially showed significant differences across different groups in morphology, social structure, behaviour, acoustics, diet, genetics, and habitat use (Baird and Stacey, 1988; Baird and Dill, 1995; Barrett-Lennard *et al.*, 1996; Ford *et al.*, 1998; Baird and Whitehead, 2000; Ford *et al.*, 2000; Scheel *et al.*, 2001; Hoelzel *et al.*, 2002). As a result the Northeast Pacific killer whales have been categorized into different ecotypes (Ford *et al.*, 2000). Compared to other marine predators killer whales have one of the most assorted diets, while prey preference depends on their ecotype; e.g. the resident ecotype of the Northeast Pacific, feeding mainly on fish, competes with other cetaceans and seals, whereas sympatric transient killer whales hunt marine mammals ranging from sea otters to other whales (Trites *et al.*, 2007). It is suggested that ecotype classification of killer whale populations from other seas should be postponed until well-founded ecological and taxonomical data have been obtained (de Bruyn *et al.*, 2013). Still, evidence exists which indicates that reassessment is needed concerning the classification of this genus; coexisting ecotypes, including ecotypes B, C and D from the Antarctic and the Northeast Pacific transient ecotype, have significantly diverged, showing distinct morphology, prey preferences, acoustics and behaviour (Morin *et al.*, 2010; Foote *et al.*, 2013).

Like all other cetaceans, killer whales rely on their underwater sounds for orientation, feeding and communication (Würsig and Richardson, 2009). Their acoustics are categorized into three main sound types, including clicks, whistles, and burst-pulse calls (Ford, 1989). These sounds are distinctive per social group (Ford, 1991).

1.7.1. Physiology

One important reason to study energetics of killer whales is that they have the highest trophic level of all marine mammals which causes their prey consumption to have potentially great impact on marine ecosystem species (Pauly *et al.*, 1998). However, the exact consequences of such an impact by these top predators can only be foreseen when their place within the ecosystem is well-known (de Bruyn *et al.*, 2013). During the last decade the impact of killer whale predation on the food-web dynamics of marine ecosystems has been heavily discussed as cause of the collapse of other megafaunal populations in the northeast Pacific (Estes *et al.*,

1998; Springer *et al.*, 2003; DeMaster *et al.*, 2006; Wade *et al.*, 2007; Springer *et al.*, 2008; Estes *et al.*, 2009; Kuker and Barrett-Lennard, 2010). Conversely, some killer whale populations are endangered, for which prey availability is suspected to play a significant role for their survival chances (Ford *et al.*, 2010; Williams *et al.*, 2011; Ayres *et al.*, 2012).

Though physiological data on killer whales is difficult (if not impossible) to collect at sea, some valuable physiological data have been collected on captive killer whales, and Table 1.3 summarizes these data for mature animals. Lenfant *et al.* (1968) studied immature specimens, yet they did calculate O_2-Hb dissociation curves and found interestingly that the whales' hemoglobin had a low affinity for O_2, so blood gives up O_2 relatively easily. Partial pressure of O_2 in the blood decreased non-linearly showing a rapid decline during the first 3 min of an apnea, while P_{CO2} increased non-linearly (Noren *et al.*, 2012b). Noren *et al.* (2012b) suggested that the initial rapid decline in partial pressure of O_2 is caused by the fact that O_2 is directly consumed by the blood cells, and simultaneously shifted from hemoglobin to myoglobin to refill O_2 store in the muscle tissues.

Non-deep diving marine mammals likely exchange gas from the lungs to the blood throughout their dive, in contrast to deep-diving marine mammals for which gas-exchange is predicted to cease at depth (Ponganis, 2011). Non-deep divers typically have greater lung O_2 stores and dive on inspiration (Wartzok, 2002; Mortola and Seguin, 2009; Ponganis, 2011), though their most important O_2 store is muscle tissue (Noren *et al.*, 2012b) (Table 1.3). Kriete (1995) examined respiratory characteristics of the killer whale most intensively and found a rather large variation in V_T for all four study animals, yet she found that only one immature specimen showed significant difference in V_T between activity states (Kriete, 1995).

Also Kriete (1995) found a significant increase in E_{O2} during each breath with increasing level of activity for all study animals (Table 1.4). She established equations for the relationship between E_{O2} and apnea per activity state for her study animals, though not all fitted well (Kriete, 1995). Noren *et al.* (2012b) revealed that an adult male killer whale (3,850 kg) had a maximum voluntary breath hold of 13.3 min while resting, close to the calculated aerobic dive limit (cADL) of 13.9 min Table 1.3. Also they found a cADL for exercising adult killer whales of 4.6 min. The maximum reported apnea observed at sea is 10.0 min (Heyning and Dahlheim, 1988).

Table 1.3. Values of physiological constants measured on captive adult male killer whales including their sources.

Feature	Data	References
Mass-specific lung O_2 ($ml \cdot O_2 \cdot kg^{-1}$)	8.1	Noren *et al.* (2012b)
Lung O_2 % from total body O_2 store	**22.6**	Noren *et al.* (2012b)
Muscle myoglobin (g Mb·(100 g muscle)$^{-1}$)	3.07	Noren and Williams (2000)
Muscle mass %	36	Goforth, unpublished data
Combining capacity Mb ($ml \cdot O_2$ g^{-1} Mb)	1.34	Kooyman (1989)
Mass-specific muscle O_2 ($ml \cdot O_2 \cdot kg^{-1}$)	14.8	Noren *et al.* (2012b)
Muscle O_2 % from total body O_2 store	**41.6**	Noren *et al.* (2012b)
Blood haemoglobin (g Hb·(100 ml blood)$^{-1}$)	14.63	Hedrick and Duffield (1991)
Blood volume ($ml \cdot kg^{-1}$)	90	Ridgway (1972)
Combining capacity Mb ($ml\ O_2 \cdot g^{-1}$ Mb)	1.34	Kooyman (1989)
Mass-specific blood O_2 ($ml\ O_2 \cdot kg^{-1}$)	12.8	Noren *et al.* (2012b)
Blood O_2 % from total body O_2 store	**35.8**	Noren *et al.* (2012b)
Combined total mass-specific O_2 ($ml\ O_2 \cdot kg^{-1}$)	35.7	Noren *et al.* (2012b)
Range P_{O2} blood (mmHg) (resting submerged)	31.0-85.0	Noren *et al.* (2012b)
Range P_{CO2} blood (mmHg) (resting submerged)	46.4-75.2	Noren *et al.* (2012b)

Still, there exists a lack of solid values on energetic requirements of killer whales. The few studies which have been conducted on this species involving direct measurements of metabolic rates are based on a small number of study animals and contain small datasets on which conclusions are drawn (Kasting *et al.*, 1989; Kriete, 1995; Worthy *et al.*, 2013). Of these studies only Kriete (1995) focused on different level of activities, while the other experiments were concentrated solely on BMR (Kasting *et al.*, 1989; Worthy *et al.*, 2013). Studies conducted on *in-situ* killer whale energetics rely on various assumptions, uncertainties and extrapolations from *ex-situ* data, which will be discussed throughout this thesis (Williams *et al.*, 2004b; 2006; Williams and Noren, 2009; Williams *et al.*, 2011).

Table 1.4. Tidal lung volumes and O_2 extraction from adult captive killer whales during different activity states. Mean E_{O2} was calculated for mean apnea. Data from Kriete (1995). (For definitions activity states see next section)

		Tidal lung volume		Mean E_{O2}
Animal	**Activity**	**Mean (L)**	**Range (L)**	**(% O_2/breath)**
Mature ♂	1	211.5	153.5-254.5	35.2
	3	205.5	82.0-258.5	47.1
Mature ♀	1	97.5	54.0-149.0	33.4
	2	78.0	50.5-114.0	38.1
	3	102.6	75.5-135.0	43.8

1.7.2. Behaviour

Killer whale behaviour at sea has often been divided into foraging, travelling, socializing and resting (Ford, 2009). Kriete (1995) measured E_{O2} for captive killer whales during different activity level. E_{O2} during "activity state 1" was measured after a 15-min resting period (Kriete, 1995). Resting free-ranging killer whales often line up next to each other and may not show significant movement forwards (Ford, 2009). At times they float at the surface, so-called 'logging'. Kriete (1995) conducted measurements for "activity state 2" and "activity state 3" after a 15-min period of moderate and high activity, respectively, during a show, involving various breaches and high speed swimming. E_{O2} for moderate activity level for males was estimated by Kriete (1995) as the average of the other two activity levels due to research limitations. At sea killer whales can reach swimming velocities up to about 8 $m \cdot s^{-1}$ during pursuit of either other marine mammals (Fish, 1998; Ford *et al.*, 2005) or large fish (Fish, 1998; Ford *et al.*, 2005; Guinet *et al.*, 2007), and take advantage of their endurance abilities while hunting either individually or in a group. During active socializing they can do aerial behaviours, such as spy hops, breaches and tail lobs and display irregular diving and surfacing patterns moving at irregular speeds in diverse directions (Williams *et al.*, 2009a). While spending time at depth killer whales normally show a general diving pattern characterised by a longer deep dive followed by a sequence of short shallow dives (Miller *et al.*, 2010). During slow travel, killer whales line up abreast and swim at steady pace in one direction while respirations become more regular and synchronous (Ford, 2009; Williams and Noren, 2009).

The activity states defined as by Kriete (1995) are difficult to apply to the behaviour of free-

ranging killer whales and it is therefore important to establish a systematic method of tracking activity level in free-ranging killer whales.

1.7.3. The Norwegian herring-eating killer whale

This study focuses on killer whales in Norway, which is the most thoroughly studied north Atlantic killer whale population. They are thought to follow the migration of herring, their predominant prey (Similä *et al.*, 1996). During summer the herring are dispersed across feeding grounds, while during winter they aggregate (Kuningas *et al.*, 2007).

Norwegian killer whales live in pods containing on average 15 individuals, showing the same matrilines and strong social organisation as north East Pacific piscivorous conspecifics (Similä and Ugarte, 1993; Beck *et al.*, 2011). However, group size varies depending on the density of herring schools; group sizes observed within the herring overwintering grounds were significantly larger due to higher herring school density compared to those of herring spawning grounds (Similä and Ugarte, 1997). Wintering herring in general occur at greater depths than spawning herring and school strongly (Sigler and Csepp, 2007; Simon *et al.*, 2007).

The killer whale ecotype included in this study displays two feeding techniques: carousel feeding, where they appear to cooperatively herd herring into a tight ball towards the surface and stun them with underwater tailslaps; and subsurface feeding, during which they show less coordination and spent less time around the herring schools (Similä and Ugarte, 1993). To maximize the efficiency of carousel feeding the whales may coordinate their actions to form a high density ball by herding the herring using low-frequency calls, air bubbles and their white body parts (Similä and Ugarte, 1993). It is suggested that one of the reasons why killer whales herd herring to the water surface could be to save energy by decreasing the distance to travel from the herring to the surface to breathe (Similä, 1997). Breathing is expected to be influenced by coordinated feeding both due to high exercise levels but also due to the fact that whales cannot all leave the herring school to surface synchronously. There seem to be no differences in diving behaviour between adult males and females during carousel feeding (Bisther and Vongraven, 1995).

1.8. Goals of the thesis research

Though captive marine mammals represent a unique opportunity to collect physiological data, their behaviour is never a true replication of their conspecifics' at sea. The development of diving models coincides with new technology, including telemetry, which enables collection of detailed information for divers at sea. Using fine-scale telemetry, diving behaviour of an animal can be evaluated directly.

For this study DTAG data was investigated, collected from *in-situ* Norwegian herring-feeding killer whales. The goal was to use fine-scale continuous data on consecutive dives, including respiration timing and underwater movements, of *in-situ* killer whales to create a simple O_2 model to reveal new and potentially more accurate data about energetics of killer whales, which simultaneously will advance the accuracy of energy uptake estimations for cetaceans.

Table 1.5. Short description of the difference in O_2 uptake for all applied and evaluated O_2 model variants during the present study

Model variant	Features	Comments
1	O_2 uptake equal for all breaths	As used in literature up to date
2	Optimized O_2 uptake equal for all breaths	
3	O_2 uptake varying per breath as function of O_2 store according to a simple O_2 uptake curve	First improvement upon fixed O_2 uptake per breath. Estimated O_2 store can never exceed estimated total body O_2 store.
4	O_2 uptake varying per breath as function of O_2 store according to an optimized O_2 uptake curve	First impression of possible true shape of O_2 uptake curve. Estimated O_2 store can never exceed estimated total body O_2 store.

In this thesis four variants of an O_2 model are presented, including different E_{O2} per breath (Table 1.5), of which the fit to the killer whale DTAG data is evaluated per individual or sex category according to the proportion of the response variable (E_{O2} per 15 min intervals) explained by the level of activity, represented either by stroking rate or speed[3]. Subsequently, these findings in relation to the different E_{O2} per breath are interpreted and discussed in the context of outcomes from previous studies, with emphasis laid on the importance of respiration timing and its influence on energetic estimates. Finally, speed and stroking rate as activity metrics applied to the O_2 model are discussed and evaluated.

2. The significance of respiration timing, in addition to frequency, in energetic estimates of killer whales

2.1. Introduction

As detailed in the General Introduction, many studies have made use of breathing rate to study metabolic rates or 'cost of transport' (COT) in cetaceans. The one crucial assumption made in these studies is that every ventilation has a constant level of E_{O2} (Sumich, 1983; Dolphin, 1987; Blix and Folkow, 1995; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014). However, there is a "family of relationships" that affects the E_{O2} during one breath, including V_T and O_2 stores at time of consecutive breaths, which in turn depend on dive duration prior to the inhalation and the MR during preceding apnea (Gallivan, 1992). Already during earlier studies on cetaceans it has been shown that E_{O2} can potentially show great variation per breathing event (Olsen *et al.*, 1969; Ridgway *et al.*, 1969; Wahrenbrock *et al.*, 1974; Sumich, 1994; Kriete, 1995; Sumich, 2001).

Ignoring the fact that O_2 uptake can vary per breath decreases the potential accuracy of breathing rate as metric for MR, which in turn potentially causes inaccurate energetic estimates from which important conclusions are drawn.

2.1.1. Respiration timing

2.1.1.1. Diving behaviour

It is foreseen that marine mammals should benefit from remaining submerged; both to maximize the time spent foraging and conducting other important activities at depth, and to minimize energy loss by surface drag and travel to the sea surface (Kramer, 1988; Thompson *et al.*, 1993; Costa and Williams, 1999). The time to reach the surface mandates the diver to take into account the time lag between decision-making and reaching the surface (Shiomi *et al.*, 2012). By making trade-offs it is likely that divers optimize their O_2 use and simultaneously optimize the way of replenishing new O_2 (timing and rate of respiration), especially during

foraging, to maximize O_2 use versus foraging success.

Kramer (1988) applied the "marginal value theorem" (Charnov, 1976) to aquatic air breathers to determine the optimal time allocation during a dive cycle including surface time related to O_2 replenishment and dive time related to energy uptake. This ground laying approach concerning the "theory of optimal breathing" has been applied in studies on many diverse divers ranging from birds (Parkes *et al.*, 2002; Wilson and Quintana, 2004; Green *et al.*, 2005; Shiomi *et al.*, 2012) to pinnipeds (Thompson *et al.*, 1993; Thompson and Fedak, 2001) to large whales (Acevedo-Gutiérrez *et al.*, 2002). Such models assume that a diver should not be fully replenished with O_2 when starting its subsequent dive (Thompson *et al.*, 1993; Walton *et al.*, 1998). Therefore the O_2 store reflects not only O_2 depletion from the most recent dive, but also from earlier apneas. On a long-time scale, V_{O2} and E_{O2} need to be balanced. On a small scale, significant deviations occur during every dive as animals deplete stored O_2 to different degrees (Goldbogen *et al.*, 2012). Though this issue was considered by (Wilson *et al.*, 2003), this factor is not taken into account in many studies on free-ranging cetaceans' metabolism.

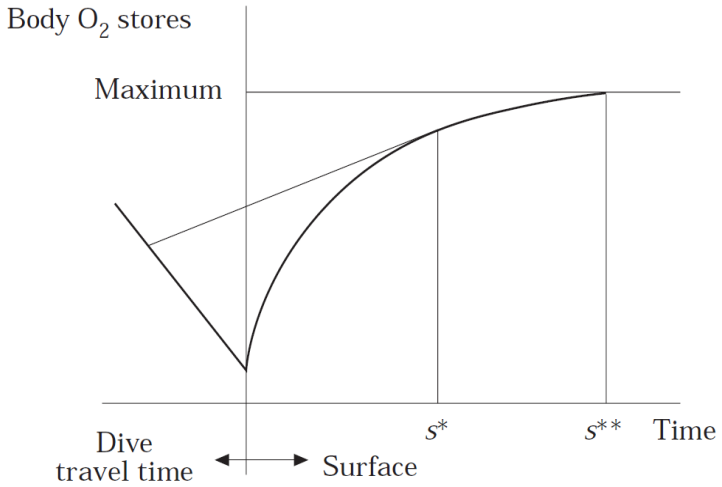

Figure 2.1. An oxygen uptake curve. Left of the origin represents the dive time, with on the right the surface interval to replenish O_2 store. *S*** represents the surface interval at which body O_2 stores would be fully replenished. *S** represents the optimal surface interval to maximize diving time, and is derived as the tangent from the dive time. The slope of the tangential represents the O_2 extraction efficiency (see text for explanation).Copied from Walton *et al.* (1998), adjusted from Kramer (1988)

The 'oxygen uptake curve' (Fig. 2.1) represents the asymptotic relationship between E_{O2} and apnea durations, or between the saturation of the total body O_2 store (TBO) and the surface interval, which would indicate the number of breaths if inter-breath intervals were constant. Net gain of O_2 per breath decreases with increasing number of consecutive breaths as O_2 stores (blood (haemoglobin), muscle (myoglobin) and respiratory tract) fill up and partial pressures of O_2 in blood become more equal to inhaled air in the lungs and alveoli, reducing the diffusion rate (Fig. 2.1) (Hampton *et al.*, 1971; Hampton and Whittow, 1976; Kramer, 1988). The slope of the tangential (Fig. 2.1) that connects a dive time and its related O_2 store after the optimal surface interval (i.e. where the tangential touches, but not crosses the uptake curve) represents the efficiency of E_{O2}: the steeper the slope the higher the efficiency. Apnea length influences partial pressure of O_2 in the body at the end of that particular dive and therefore the efficiency of E_{O2} from inhaled air; a prolonged apnea causes a larger depletion of O_2 storage compared to shorter dives. The efficiency increases more swiftly for short dives and flattens for longer dives when the maximum possible uptake is approached (Hampton and Whittow, 1976; Kramer, 1988; Kasting *et al.*, 1989; Thompson *et al.*, 1993; Sumich, 1994; Kriete, 1995; Sumich, 2001; Parkes *et al.*, 2002). A higher MR, dependent upon activity level, causes the O_2 store to deplete faster than a lower MR. Therefore a higher MR causes a greater O_2 flux, and so higher E_{O2}, compared to a lower MR after an equivalent breath interval. It has been hypothesized that a surface interval initially will include longer breath intervals to maximize E_{O2}, which throughout the breathing bout will shorten to maximize CO_2 removal (Dolphin, 1987; Wilson *et al.*, 2003).

Different behaviours have different functions (foraging, travelling, resting, socializing, fleeing etc.) and influence swimming modes, or MR, and therefore respiration timing (Fedak and Thompson, 1993; Wartzok, 2002). It is thought that cetaceans display wave-riding and porpoising as a strategy to save energy at high swimming speeds (Williams *et al.*, 1992; Williams, 2001). Depending on a situation, an animal simultaneously makes both physiological and behavioural decisions, driven by the present situation and the near future, which will shape their diving and breathing behaviour (Fedak and Thompson, 1993; Thompson *et al.*, 1993).

2.1.1.2. The aerobic dive limit versus the O_2 balance

Though it is unfeasible to estimate TBO without making assumptions (Ponganis *et al.*, 2011), it is an important starting-point for assessing respiration timing in divers and is often estimated using the allometric equation established by (Kooyman, 1989), which is modified for odontocetes by Shaffer *et al.* (1997) and Noren *et al.* (2002). TBO is spread over the respiratory tract, blood and muscle (Williams and Worthy, 2002).

The ADL or diving lactate threshold (DLT) is the "dive duration associated with the net increase in whole body lactate, presumably resulting from anaerobic energy production following O_2 depletion in some tissue" (Kooyman *et al.*, 1980; Butler and Jones, 1997). This ADL is considered to reflect the TBO capacity and the rate at which these are consumed (MR) (Kooyman, 1989). ADL can differ within and between species and even within an individual, caused by variation in O_2 storage ability, depending on various physiological features, and differences in diving MR (Kooyman, 1989; Noren *et al.*, 2002; Green *et al.*, 2007; Villegas-Amtmann and Costa, 2010; Noren *et al.*, 2012b). Large marine mammals are predicted to have greater ADLs because of their absolute size of available O_2 stores in combination with their relatively low MR per body mass (Schmidt-Nielsen, 1997; Noren and Williams, 2000; Costa, 2009; Noren *et al.*, 2012b).

For some studies on aerobic divers, reaching the ADL was assumed to be the primary cue for respiration (Kooyman *et al.*, 1980; Kooyman, 1989; Ponganis *et al.*, 1993). However, accepting the fact that ADL is not a constant, as stressed above, applying a (calculated) ADL or DLT as diving threshold would be an "oversimplification of a complex system" (Green *et al.*, 2007). Above all, Houston (2011) brought up the crucial common misconception about the difference between ADL (diver uses all the O_2 that it CAN store) and "oxygen balance" (diver uses all the O_2 that it HAS stored). This misconception potentially explains the fact that for many studies it was concluded that divers surfaced (well) within their capability constraints (Martin and Smith, 1999; Otani *et al.*, 2001; Miller *et al.*, 2010; Houston, 2011). Furthermore, it is not realistic to assume that a diver fully depletes its O_2 stores; vital organs such as the brain and central nervous system need O_2 to function (Butler, 2006). A diver is expected to have a "safety margin" to accommodate any unforeseen apnea elongation, without it being fatal (Wilson and Quintana, 2004; Houston, 2011). Still, it is expected that divers use oxygen balance strategies,

depleting their O_2 stores vastly, to maximize E_{O2} (Kramer, 1988; Houston, 2011). In summary, it is expected that marine mammals "begin a dive with optimally loaded O_2 stores, and resurface when these are optimally depleted", rather than relating surfacing to ADL (Thompson *et al.*, 1993).

Over a sufficiently long-time scale, V_{O2} and E_{O2} need to be balanced. On a shorter time scale, significant deviations are expected during every dive as animals deplete stored O_2 to different degrees, and anaerobic divers may even increase O_2 debt by temporarily switching to anaerobic metabolism which accumulates lactate (Horning, 2012). (Sumich, 1994) identified the fundamental conclusion that respiration timing influences E_{O2} by marine mammals, stating that "breath interval can serve as a reasonable basis for estimating E_{O2} and, in combination with observed breathing rate and estimates of V_T, will contribute to the development of an improved understanding of the metabolic activity". Subsequently, also Kriete (1995) suggested according to her results that "respiration rates and metabolic rates are not related linearly to each other; hence by just collecting respiration rates in marine mammals, energy demands cannot be deduced directly from respiration rates". The aim of this present study is to more fully evaluate the potential influence of respiratory timing on estimates of metabolic rates.

2.1.2. Metabolic rates, speed and cost of transport

Making quantitative predictions concerning respiration timing of a diver also involves estimation of the MR during the dive phase, an aspect which is commonly simplified by using a constant MR (Thompson *et al.*, 1993; Houston, 2011). V_{O2} during a dive potentially has influence on the expected E_{O2} per breath, as explained above. Shiomi *et al.* (2012) revealed the importance of variation in MR from exercise, finding that stroke rate was a better predictor of surfacing timing than dive duration in emperor penguins. Constant swimming speed is expected to be energetically most efficient. However, constant swimming is a rarity, for marine mammals adjust their swimming mode and speed almost constantly according to their behavioural state on fine time scales.

To measure speed accurately one needs to know the total distance travelled, which is unfeasible to determine from surface observations alone. Surface estimates of speed are based upon the minimum possible travel distance between two surfacing points (Sumich,

1983; Kriete, 1995; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009) and could therefore have resulted in an overestimation of the MR per unit distance. For this chapter this same activity metric, speed, derived from continuous fine-scale telemetry data, was implemented in the O_2 model as reflection of activity level, or MR.

By dividing the 'mass-specific MR' by the swim speed of an animal one can calculate the COT, which indicates the MR for moving a unit mass over a unit distance (Costa and Williams, 1999). The COT includes the BMR as a component of the total MR, and is used to compare energetic needs of animals within or between species (Costa and Williams, 1999). COT for killer whales was previously estimated by Kriete (1995) and Williams and Noren (2009).

The fact that over a sufficiently long time-scale V_{O2} and E_{O2} should balance provides a tool whereby predicted levels of E_{O2} can be assessed. The goal of this chapter was to create an O_2 exchange model, allowing variation in O_2 uptake per breath, in order to derive realistic estimates of energy requirements over 15 min intervals from longitudinal observations of breath times and underwater activity levels of free-ranging killer whales. By including continuous data on respiration timing and underwater exercise, both recorded by an animal-attached tag, a model of O_2 store enabled a re-examination of E_{O2} dynamics. Using this model the objective was to investigate the potential influence of respiration timing, in addition to respiration frequency, on energetic estimates for killer whales at sea. According to the results it is discussed whether respiration rate alone is an accurate reflection of underwater activity, and therefore MR, in free-ranging killer whales. Also, the fixed O_2 uptake value per breath used for making *in-situ* killer whale energetic estimations by Williams and Noren (2009) will be investigated.

2.2. Material and methods

2.2.1. Data collection

The data used for this study were collected during the 3S project; for detailed information on data collection and other information on this project see the technical report by Miller *et al.* (2011). 12 Adult herring-feeding killer whales were instrumented with suction-cup attached

DTAGs (Johnson and Tyack, 2003) during the winters of 2005 and 2006 and the summer of 2009 along the northern Norwegian coast between 67° and 69° northern latitude in the waters of the Vesfjord/Ofotfjord/Tysfjord-area (Miller *et al.*, 2011) (Table 2.1, Fig. 2.2). Whereas during 2005 and 2006 the animals were tagged within the inside waters of the Lofoten islands, in 2009 two individuals were tagged in offshore waters on the continental shelf break on the outside of the Lofoten islands (Fig. 2.2). During tag deployments information on the characteristics of the tagged individual was collected, including gender, calf presence, behaviour, group size and distinct behavioural events. Adult male killer whales were easily recognized by their tall dorsal fin (Ford, 2009). Other adult-sized animals were scored as adult female; though it is possible some of them may have been juvenile males. Still, these individuals had a smaller body size than adult males, which was the physiological underlying reason for gender categorization, rather than the gender itself. Individuals categorized as calves, juveniles or sub-adults, and individuals for which age and sex were not determined, were excluded from the present study.

Table 2.1. Information on tagging deployments of adult killer whales included in analyses during the present study including whale ID, date, gender, position of tag deployment, and length of tag record

Whale ID*	Date	Sex	Latitude	Longitude	Length record (hh:mm:ss)
05_316a	12-11-2005	F	68°16.22'N	16°05.60'E	2:39:35
05_320a	16-11-2005	F	68°15.35'N	16°05.92'E	4:42:00
05_320b	16-11-2005	M	68°15.09'N	16°11.95'E	2:12:00
05_321b	17-11-2005	F	68°15.66'N	16°12.03'E	1:40:44
05_322a	18-11-2005	F	68°12.93'N	16°13.52'E	3:35:19
05_322b	18-1-2005	M	68°11.51'N	16°24.18'E	3:03:40
06_313s	9-11-2006	F	68°13.71'N	14°53.74'E	2:11:59
06_314a	10-11-2006	M	68°20.11'N	15°54.75'E	2:04:47
06_327s	23-11-2006	F	68°00.01'N	13°40.81'E	5:25:30
06_327t	23-11-2006	M	68°00.18'N	13°40.39'E	5:41:44
09_144a	24-5-2009	M	68°26.73'N	12°37.04'E	11:48:00
09_144b	24-5-2009	M	68°27.26'N	12°38.51'E	12:28:33

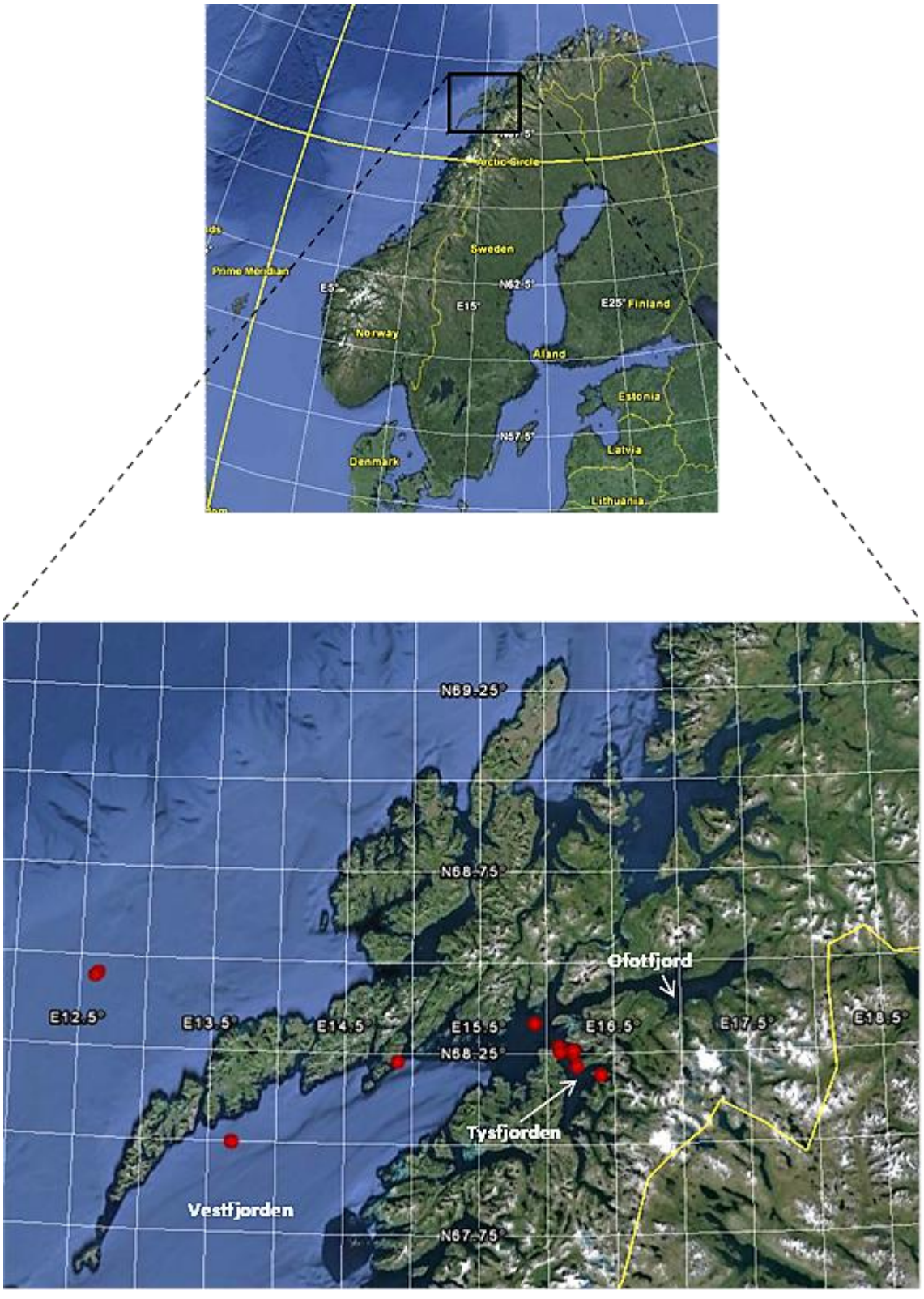

Figure 2.2. Map of field site. Top: Scandinavia with the box corresponding to zoomed-in map. The yellow horizontal line represents the Arctic Circle. Bottom: Lofoten area including the Vesfjorden, Ofotfjord, and Tysfjord. Red dots indicate the coordinates of tagging events of individual killer whales. (Projection: Simple Cylindrical with WGS84 datum)

2.2.2. Tag data processing

DTAG data were analysed using MATLAB® (version 7.5.0.342R2007b - Mathworks) and RStudio® (version 0.98.994 - The R Foundation for Statistical Computing). Continuous acoustic and sensor recordings of the DTAG are synchronous, so the relative timing of sounds and motion can be determined precisely. Prior to running the analyses, sensor tag data, including pressure, magnetometer - and accelerometer data, were calibrated and condensed to a sampling rate of either 10 Hz for tags deployed in 2005 and 2006, or 5 Hz for tags deployed in 2009. Pitch, roll and heading data were generated by converting raw orientation measurements from the magnetometer and accelerometer to scientific units in micro-Tesla and g, respectively, according to the tag axes. Finally, tag position on the whale (which is sensitive to change during deployment) was accounted for by correcting the three-dimensional axes with vectors derived from the tag orientation during surfacings (Johnson and Tyack, 2003).

2.2.3. Respiration events

High-resolution depth sampling using DTAGs enables detection of individual surfacings, which are breathing events in this single breathing species (Miller *et al.*, 2010) (Fig. 2.3).

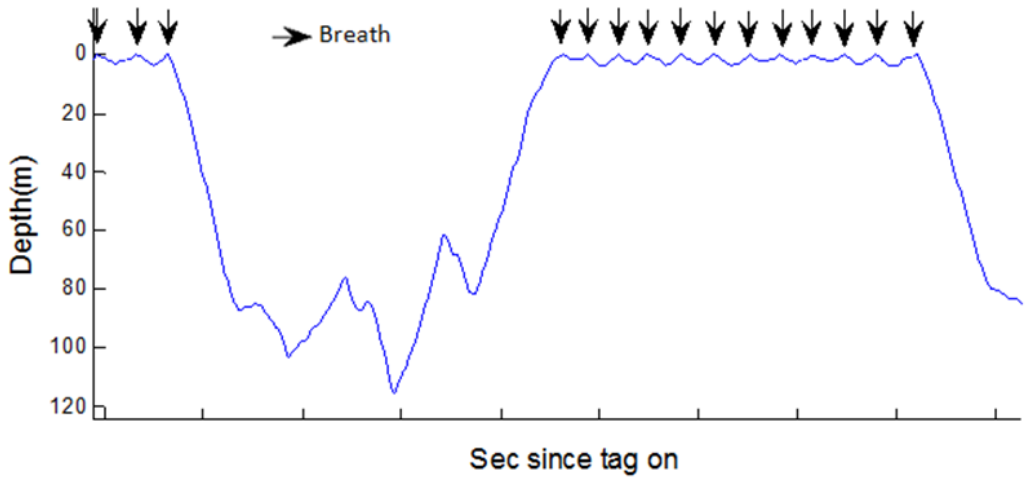

Figure 2.3. Example of a time series of a dive profile and the visualization of breathing events (black arrows).

To extract respirations for all tagged individuals, pressure measurements from the DTAG data were converted to depth records in metres by calibration. First, these depth data were analysed automatically via surfacing cues using the "*findsurfs*"-function in MATLAB® (Johnson, unpublished data) to extract surfacing events per tag record. Within this function a surface parameter was implemented as a threshold in meters for detecting a surfacing. This threshold differed per individual (ranging from 0.3 to 1.1 m) depending on the tag placement location on the body of each whale. The detected surfacings were then manually checked to confirm them as breaths by investigation of the dive profile (Fig. 2.3), and, in case of uncertainty, the acoustic recordings. When a respiration was suspected, yet not automatically detected, often it could be confirmed through either water sounds and sometimes the blow sound itself. The DTAG data also included "*roll*" indication which was measured through 3-axis magnetometers and showed whether the individual was up-right during a surfacing; if the whale was not up-right it could not have taken a breath with its blowhole underwater.

2.2.4. Speed measurements from flow noise

As the DTAG does not contain a speed sensor, speed was estimated by merging analyses of kinematic measurements and flow noise, recorded simultaneously and continuously with the DTAG. Flow noise is combined with sensor data since speed estimation from kinematics is only accurate during ascents and descents, when the whale has high body pitch angles. The greater the body pitch angle, the closer speed through the water can be estimated using the rate of change of depth.

Speed was derived from the sound pressure levels (SPL's) of the recorded low-frequency flow noise, as done previously by other authors (Goldbogen *et al.*, 2006; Simon *et al.*, 2009; Goldbogen *et al.*, 2012; Simon *et al.*, 2012), per individual whale to account for tag placement, and per tag period to account for tag movements. Acoustic data were uploaded and sub-sampled from 96 kHz to 1 kHz to reduce the size of the data for analyses. A 4^{th} order Butterworth low-pass filter was implemented to create a sharp frequency cut-off and have a flat frequency response. The frequency of the low-pass filter varied from 200 to 350 Hz between individuals and was specified per tag record so that the fluctuations in flow noise, within individual records, were captured most effectively. Root mean squared (RMS) levels, with an averaging window of 50 ms, were computed from the squared band-pass filtered

pressures using the "*filtfilt*"-function in MATLAB® to eliminate a phase shift. These were ultimately converted into SPL's in µPa, which had the same sampling rate as the sensor data (50 Hz).

Speed through the water was estimated kinematically for all entire tag records by using the "*vertical*"-function in MATLAB® (Johnson, unpublished data) to calculate the rate of depth change. The depth change rate over 1s intervals was divided by the sine of the body pitch angle:

$$(d_t - d_{t-1}) / \sin \alpha_t, \quad (3)$$

Where d_t is the depth at second t, d_{t-1} is the depth at the preceding second, and α_t is the body pitch angle at second t.

The SPL's from the flow noise and the kinematic speed estimates from the sensor data were synchronized over 1s intervals. A pitch angle threshold was introduced which varied per individual and per tag period depending on data quantity and pitch angle occurrence and distribution. The lowest minimal pitch angle used was + 45°/ - 45°, which seemed realistic compared to previous studies (Miller *et al.*, 2004; Ware *et al.*, 2011). The highest minimal pitch angle used was + 65°/ - 65°, equal to the one used by Goldbogen *et al.* (2006). As surfacings produced high flow noise levels as well, unrelated to swimming speed, a depth threshold of 10 m was implemented to eliminate intervals at the surface.

After eliminating surfacing intervals and intervals with small body pitch angles an exponential least-squares fit between the flow noise SPL's and speed through water was established per tag record and tag period within records using regression analyses, using the following equation:

$$U = a * \exp (b * \mathrm{SPL}), \quad (4)$$

Where, U is the speed through water in $m \cdot s^{-1}$, a and b are estimated coefficients, and SPL is the sound pressure level in dB re 1 µPa calculated from the flow noise. Other authors which conducted studies on speed estimations from flow noise foregoing this study mostly chose for

a polynomial function (Goldbogen *et al.*, 2006; Goldbogen *et al.*, 2008; Simon *et al.*, 2012). However, during this study an exponential function was chosen as regression between SPL and speed through water particularly since a polynomial curve would result in an unrealistic increase in speed estimation for SPL values lower than the SPL value that gives the lowest speed estimation. The parameters for this exponential function were determined per individual whale and per tag period within individuals. To define valuable correlations there had to be a considerable amount of data points (depending on spreading) covering a range of at least 10 dB. In the occurrence of a gradual tag movement, an average correlation was determined from the previous and following tag period. When for one of these periods it was not feasible to determine a speed-SPL correlation, the gradual movement period was handles as a normal tag period if feasible. For tag periods for which it was not feasible to determine the speed-SPL correlation, the correlation of the preceding period was maintained. These established relationships were then used to calculate the continuous speed through the water from the recorded flow noise level per one-second intervals for the entire tag records.

To take into account speed overestimation by the increase of the SPL caused by breaking the surface, it was investigated whether it was more sensible to take either a time criterion around a breathing event or a depth criterion; it appeared that the time criterion was more accurate in accounting for SPL difference caused by surfacing. Accordingly, the speed measurements of the three last seconds before the breath and at time of the breath were replaced by the measurement of the fourth second before the breath. The three succeeding measurements after the breathing event were replaced with the speed value of the fourth second after the breathing event.

Consequently, time series data of speed for all individual whales were investigated for outliers through evaluating the difference between consecutive 1s measurements. The difference threshold was set at 2.8 $m \cdot s^{-1}$ after which the number of observations showing a greater deviation than this decreased substantially. When a speed measurement showed a greater deviation from the previous or succeeding measurement than this criterion, the measurement was considered an outlier and replaced by the mean speed of that entire tag record. One female (06_327s) and three males (06_327t, 09_144a and 09_144b) showed outliers, with a minimum of one (06_327s, 06_327t, and 09_144b) and a maximum of four (09_144a) per tagging record.

For one male no audio was recorded, while for one female there were insufficient periods with sufficient pitch angles to derive kinematic speed. Both of these individuals were excluded from speed analyses.

2.2.5. Metabolic cost in relation to speed

The total MR (MR_T in $LO_2 \cdot s^{-1}$) of the swimming whales was estimated by the accumulation of the BMR (in $LO_2 \cdot s^{-1}$) and locomotion costs (MR_L in $LO_2 \cdot s^{-1}$) per second:

$$MR_T = BMR + MR_L, \quad (5)$$

BMR was used as quantified by Kriete (1995), who measured standard metabolic rates (SMR), herewith assumed to be comparable to BMR, for a captive adult male and female killer whale of 0.105 $LO_2 \cdot s^{-1}$ and 0.0731 $LO_2 \cdot s^{-1}$, respectively.

To found a relationship between speed and energetic cost, for both adult male and female killer whales, a function was written comprising equations as used previously by Fish (1998) and Guinet *et al.* (2007). By implementing these equations MR_L was estimated according to the mean thrust power (P_T, W) needed to overthrow the drag, caused by movement through the water related to speed, and considering the propulsive efficiency (η).

The Reynolds number (Re) was included to take into account the flow of sea water around a whale's body while swimming, depending on the length of the animal (L, in m), the swimming speed (U, in $m \cdot s^{-1}$), and kinematic viscosity (v) of sea water ($1.044 * 10^{-6}$ $m^2 \cdot s^{-1}$):

$$Re = LU/v \quad (6)$$

A relationship between Re and the drag coefficient C_D exclusively for killer whales, provided by Fish (1998), was used to estimate C_D as done previously by Guinet *et al.* (2007).

$$C_D = 6.35\ Re^{-0.3719} \quad (7)$$

P_T, which is expected to increase as a cubic function of speed (Kooyman, 1989), was estimated

through the following equation as proposed by Fish (1998):

$$P_T = 0.5\ \rho\ SU^3C_D \quad (8)$$

Where, ρ is sea water density (1,026 $kg \cdot m^{-3}$), and S is the body surface area in m^2. Consequently, to estimate the ultimate MR_L, the P_T was corrected for propulsive efficiency (η) to account for active drag caused by body movements while swimming:

$$MR_L = P_T/\eta, \quad (9)$$

With η set at 0.8 as an average propulsive efficiency for killer whales, which was determined by Fish (1998) (Fig. 7).

The goal was for the O_2 store in the model to fluctuate per 1s intervals according to the V_{O2} and thus the level of activity of the whale, here quantified as $speed^3$. To quantify the relationship between MR_L and the activity metric A ($speed^3$) for 1s intervals, Eq. 9 was rewritten including coefficient k:

$$MR_L = k\ A, \quad (10)$$

The relationship between $speed^3$ and MR_L was determined using morphometrics for either an adult male or female killer whale into the function for which values on surface area (S) and body length (L) were used presented by Fish (1998), and the body mass from Kriete (1995) (Table 2.2). Consequently, the function was run for a speed of 0 to 10 $m \cdot s^{-1}$, and a positive linear curve was fitted for both males and females (Fig. 2.4). The slope of the curve was calculated, while the intercept was set at BMR (Fig. 2.4). Due to uncertanties in the slope of MR_L vs. $speed^3$ (k) this value was varied over a range of values (Table 2.2) in a sensitivity analysis.

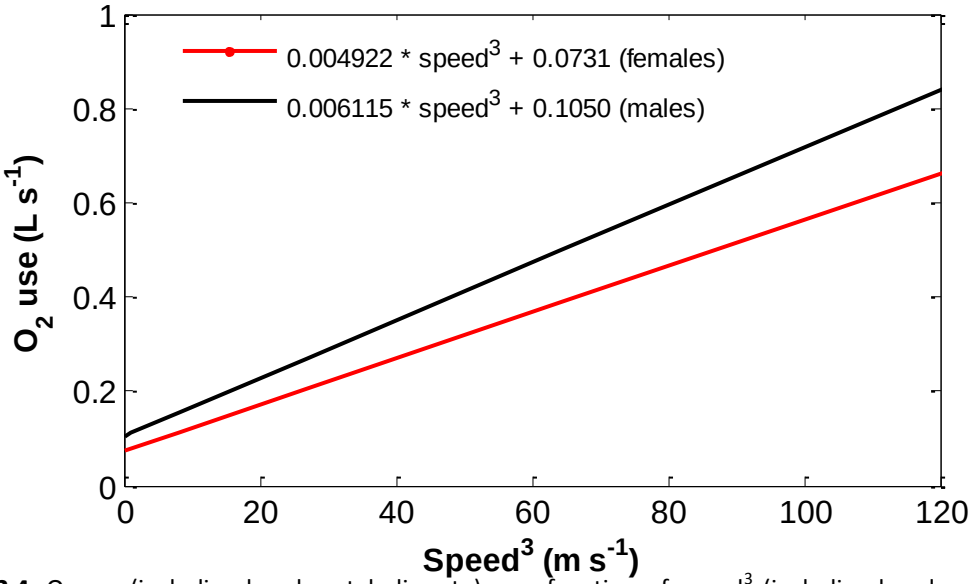

Figure 2.4. O_2 use (including basal metabolic rate) as a function of speed^3 (including basal metabolic rate) for adult female and male killer whales as implemented in the O_2 model. Assumedly, males have a higher cost related to speed in comparison to females due to higher drag caused by a bigger body size.

Table 2.2. Values of all parameters used for the O_2 model for both males and females

		Value		
Parameter	**Unit**	**Male**	**Female**	**Source**
Body length	m	6.65	5.40	Kriete (1995)*
Body weight**	kg	3,913	2,800	Kriete (1995)
Body surface area	m^2	22.90	17.00	Fish (1998)*
Lung O_2 store	L	31.1	22.7	Noren *et al.* (2012)
Muscle O_2 store	L	57.0	41.4	Noren *et al.* (2012)
Blood O_2 store	L	49.2	35.8	Noren *et al.* (2012)
Total body O_2 store***	L	137.3	99.9	
BMR	$LO_2 \cdot s^{-1}$	0.1050	0.0731	Kriete (1995)
Fixed O_2 uptake	$L \cdot breath^{-1}$	22.3	11.9	Kriete (1995)
Max O_2 uptake/breath	$L \cdot breath^{-1}$	25.52	13.68	Kriete (1995)
Slope of MR vs. speed^3	$LO_2 \cdot m^{-1} \cdot s^{-1}$	0.006115	0.004922	Present study, Figure 2.4
Slope range in sensitivity analyses	$LO_2 \cdot m^{-1} \cdot s^{-1}$	0.0031 - 0.1695	0.0025 - 0.1215	

* Extrapolation of data from Kriete (1995) or Fish (1998)
** Body mass was estimated by Kriete (1995) for her study animals using the equation by Bigg and Wolman (1975)
*** Total body oxygen store (TBO) was estimated through accumulation of maximum lung, muscle, and blood O_2 store capacities

2.2.6. The O_2 Model

To estimate each individual's O_2 store dynamically in time, by which the E_{O2} is estimated per breath, an O_2 exchange model was established combining activity level indicators from the tag data with existing information on killer whale physiology and energetics (Table 2.2):

$$O_2\ store_t = O_2\ store_{t-1} - V_{O2\ t-1} + E_{O2\ t} \quad (11)$$

where, O_2 store represents the total O_2 stored (L) in the lungs, blood and muscle together at time t (or t-1) in seconds, $V_{O2\ t-1}$ is the O_2 (L) estimated to have been used according to the MR (Eq. 10, including the BMR) estimated from speed measured during the preceding second, and $E_{O2\ t}$ is the O_2 (L) taken up through breathing at time t. E_{O2} was specified as either a fixed E_{O2} or from an O_2 uptake curve if there was a breath in the interval, and was zero otherwise.

The model was initialized at the first second after the first breathing bout of at least six breaths in the tag record, at which point O_2 stores were assumed to be saturated; the TBO was assumed to reflect the combined lung, blood, and muscle O_2 capacity. For every second thereafter O_2 store was estimated, depending on accumulating V_{O2} and whether the whale took a breath or not. If the whale remained submerged, O_2 store decreased. However, for every second the whale respired, the O_2 store was increased with an uptake amount depending on the degree of O_2 store saturation at the time of the breath. Because the relationship between O_2 uptake per breath and the O_2 store at the time of each breath was the unknown in this model, the model was implemented using alternate O_2 store – O_2 uptake relationships.

V_{O2} estimates, which are the key product of the model, were produced through averaging the E_{O2} over 15 min intervals, as will be explained in Section 2.2.7.

2.2.6.1. The O_2 model using fixed O_2 uptake per breath

For all studies on *in-situ* cetacean energetics upon to date which used breathing rate alone (Sumich, 1983; Dolphin, 1987; Blix and Folkow, 1995; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014), breathing rate is multiplied with an

average E_{O2} value, which is either measured from animals in captivity, derived through extrapolations of data on other species or a combination of existing data. A conversion factor of 20.1 $kJ \cdot LO_2^{-1}$ or 4.83 $kcal \cdot LO_2^{-1}$ is used, and a percentage of 20.95 of O_2 in air, to produce MR estimations. During the present study the O_2 store at time t, implying a constant O_2 uptake per breath (E_{O2f}) as used in existing literature, was estimated using the following equation:

$$O_2store_t = O_2store_{t-1} - (BMR + MR_L) + (b_t * E_{O2f}) \quad (12)$$

Where b_t represents a potential breathing event at second t and can take the value of either 1 (breath) or 0 (no breath).

Kriete (1995) found a mean E_{O2} for all three investigated activity states together of 41.15 % and 38.10 % of max V_T for males and females, representing an E_{O2} of 22.3 and 11.9 LO_2 per breath respectively (Table 2.2). These values were afterwards used by Kriete (1995) and Williams and Noren (2009) to estimate energetic requirements for free-ranging killer whales related through speed measured during a theodolite study.

2.2.6.2. The O_2 model using an optimized fixed O_2 uptake

Since the oxygen balance model introduced in the previous section is based on constant E_{O2} values measured directly from one adult male and female killer whale, it was not theoretically correct to compare this model with the new models implementing a fluctuating E_{O2}, for the latter one depends on the application of different parameters (O_2 store, (B)MR, etc.). Therefore, an O_2 model was created, including a fixed E_{O2} estimated according to the same parameters as applied to the models with fluctuating E_{O2}.

Fixed E_{O2} amount were manually optimized by the accumulation of the V_{O2} over the entire tag record of each whale divided by the number of breaths taken by that individual. This way a fixed uptake value was derived per individual and per sex category which inflicted a balance between the overall V_{O2} and E_{O2} at the end of a record.

2.2.6.3. The O_2 model including a set O_2 uptake curve

A key feature of this model was the inclusion of the features of a set O_2 uptake curve (see Fig. 2.1), so that E_{O2} could vary per breath and be dynamically estimated based upon modelled O_2 store at the time of each breath. This uptake curve specifies that the E_{O2} can never cause the O_2 store to exceed its full capacity. When O_2store_{t-1} was smaller than the estimated TBO (O_2store_{full}) minus the maximum possible E_{O2} (E_{O2max}), then:

$$O_2store_t = O_2store_{t-1} - (BMR + MR_L) + (b_t * E_{O2max}) \quad (13)$$

Otherwise,

$$O_2store_t = O_2store_{t-1} - (BMR + MR_L) + (b_t * (O_2store_{full} - O_2store_{t-1})) \quad (14)$$

E_{O2max} values per breath per sex applied in this model were derived from data collected by Kriete (1995), who measured the mean E_{O2} (using maximum V_T of 258.5 L and 149.0 L for males and females, respectively) during high activity level for mean apnea intervals (Table 2.2). E_{O2} is a function of the O_2 store at the time of each breath as presented in Fig. 2.5.

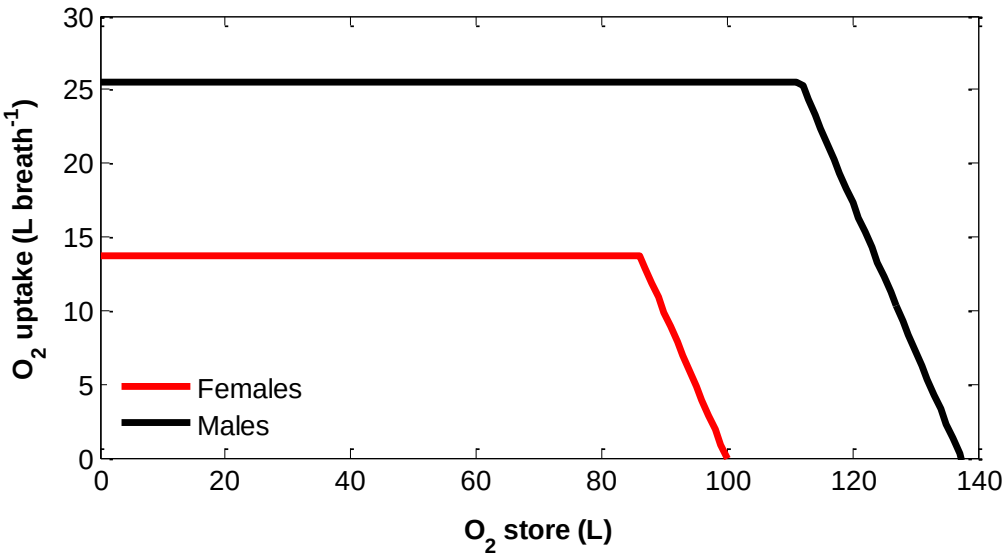

Figure 2.5. A set O_2 uptake curve for males (black) and females (red) as used in the O_2 model, wherein O_2 uptake is a function of the O_2 store at the time of each breath. The maximum O_2 uptake per breath was set at 25.52 L and 13.68 L, for males and females, respectively. The total body O_2 store capacity was set at 137.3 L and 99.9 L, for males and females, respectively.

2.2.6.4. The O_2 model including an optimized O_2 uptake curve

Optimization of the O_2 uptake curve was conducted, assuming the curve took the form of a decreasing density dependent curve. A density dependent curve was chosen for it was expected that E_{O2} per breath would be maximum when the O_2 store was fully depleted, while decreasing non-linearly with greater O_2 stores:

$$O_2store_t = O_2store_{t-1} - (BMR + MR_L) + (b_t * (E_{O2max} * (1-(O_2store_{t-1} / O_2store_{full})^X))) \quad (15)$$

where, *X* is the exponent that shapes the density dependent curve, which was optimized, per individual, sex category and all animals together. Using this model the E_{O2} per breath fluctuated according to O_2 store at the time of a breath, which in turn was defined by the preceding breath interval and level of activity during that apnea.

Exponent *X* in Eq. 15 was optimized per individual killer whale, sex category and for all individuals together using the one-dimensional optimization function "*optim*" in RStudio®, applying the "*Brent*"-method, which allowed one to define the lower- and upper bound in which to search for the optimal value of *X*. During optimization BMR was neglected since this was a parameter set as a constant value in the model depending on sex. BMR was extracted from the 15 min block E_{O2} measurements before running the optimization, so that the correlation between O_2 uptake and speed would only comprehend locomotion costs. Therefore, the intercept of this correlation was set to go through the origin during optimization (unless mentioned differently). Doing so, the exponent *X* was revealed which resulted in the maximum coefficient of determination, or r^2 statistic, representing the tightest correlation between E_{O2} and MR_L over 15 min blocks, and so made the model fit best the data.

Similar to the O_2 uptake curve applied in Eq. 14, the curve included in Eq. 15 reflected the E_{O2} as function of the O_2 store at the time of each breath. When the O_2 store was fully depleted, the E_{O2} was maximal. As the store was replenished, the E_{O2} decreased non-linearly. When the O_2 store was fully replenished, it was assumed there was no E_{O2}. From a physiological view, it was hypothesized that exponent *X* could not take on a negative value.

2.2.7. Time intervals for contrasting whale activity and O_2 extraction

Both Kriete (1995) and Williams and Noren (2009) established energetic estimations over observation tracks of at least 10 min or 15 min, respectively. This time duration is sufficient to smooth out O_2 store fluctuations during typical dives, but short enough to capture variation in underwater activity levels. To be able to compare findings from these two studies on killer whales with outcomes from the present study, speed, breathing rate, and E_{O2} (all measured or estimated per second to apply to the model) were averaged over the same 15 min blocks within each individual.

The '*filtfilt*'-function in RStudio® ("*signal*"-package) was used to average the 1s interval measurements of speed, breathing rate, and E_{O2} with a zero-phase filter over 15 min time windows or blocks. The first block started at the first second after the first breathing bout of at least six breaths in the tag record, for which the total O_2 store was assumed to be saturated. Successively, only the midpoint values of these blocks were extracted to model estimated E_{O2} over the same 15 min blocks against level of activity. However, during "*filtfilt*"- filtering the first and last filtered points, or in this case 15 min block midpoints, are not a true reflection of the data due to a (n-1)/2 delay (n = filtering window), which is padded as zeros to create a full window length over which to filter. Hence, to eliminate potential influence by start and stop points both the first and last 15 min blocks were not used in further analyses.

2.2.8. Statistical modelling and analyses

2.2.8.1. Statistical model design and model criteria evaluation

Impact of V_{O2} on either breathing rate or E_{O2} over the 15 min blocks, was investigated through regression analyses, using speed^3 as the explanatory variable. It was hypothesized that V_{O2}, or level of activity, and E_{O2} should be balanced over the 15 min blocks for short-duration diving killer whales, with E_{O2} increasing as a linear function of speed^3 (Kooyman, 1989).

Speed data were tested for normal distribution by plotting frequency distribution histograms of model residuals. Consequently, since residuals did not show a normal distribution,

application of Generalized Linear Models (GLMs) was chosen over normal Linear Regression (LM) models to evaluate the fixed effect of activity level over the 15 min blocks on the respiration rate and E_{O2} of the corresponding 15 min blocks per whale. Because of the linear response data the GLM was equipped with a Gaussian, or normal, distribution with "*identity*"-link function to provide the relationship between the variables and outcome, which is the appropriate link function for a Gaussian distribution if the variance is known (Mildenhall, 1999). To validate the application of GLMs, criteria regarding this model design were tested per individual whale before performing statistical analyses. Given the symmetrical and constant variance of the residuals in the same plot it was determined that there was indeed a linear relationship between predicted E_{O2} and speed^3 for all individuals. Normality in the residuals was confirmed, after implementing a Gaussian family with "*identity*"-link function, based on a normal-quantile plot, a.k.a. QQ-plot. Homogeneity of the residual variance, another criterion for using a GLM, was examined through plotting the model residuals versus the predicted values to detect potential residual trends. An important criterion was independence of data points within each whale. This was inspected by visualizing the distribution of the model residuals to check whether there existed a pattern in the residuals versus time. Also, a *lag*- and an auto-correlation plot ("*acf"*-plot) of the residuals were produced to envision the estimated auto-correlations between time lags. Moreover, a LM was fitted to the residuals (i) versus residuals (i-1) which would result in a strong correlation and therefore a high r^2 - and a low *p*-value if auto-correlation existed.

Due to detected auto-correlated data for at least one female (05_316a) it was chosen to apply a generalised estimating equation generalised linear model (GEEGLM, "*geepack*"-package, RStudio® version 0.98.994) for all individuals with correlation structure "*AR(1)*" to account for auto-correlation which exponentially decayed over time.

Also for category analyses a GEEGLM was used to account for random effects by individual whales, treated as clusters, within these categories. Since 15 min block speed measurements were more similar within individuals than between individuals (regardless of order), the "*exchangeable*"-correlation structure was applied to the GEEGLM. Similar to the model applied for the individuals killer whales explained above, GEEGLM applied per category was equipped with a Gaussian family and "*identity*"-link function. It was chosen for a GEEGLM over a normal GEE for this latter type of model did not account for data gaps in time series, while time series

lengths differed per individual.

2.2.8.2. Goodness of fit evaluation

All models were validated per individual or category by evaluating the strength of the correlation between predicted E_{O2} and level of activity. Though BMR inclusion in the O_2 model was necessary to make more accurate estimations for O_2 store at time of each breath, BMR was subtracted from the E_{O2} estimates during model analyses, so that the intercept of the model was set to go through the origin and only locomotion costs versus activity level were fitted. Because the main purpose of the models was prediction, r^2 values were seen as the most valuable regression statistics to optimize and compare, for they indicate how accurate the model predictions are compared to the observed data or how much of the total variation in the response variable (predicted E_{O2}) was explained by the explanatory variable (speed^3). A better fit of a model to the data within or between individuals or categories was indicated by a higher r^2 value.

Only the O_2 model including the optimized O_2 uptake curve was analysed for all whales together, where after the result was implemented per sex category.

2.2.9. Sensitivity Analyses

Whereas the variables which were implemented in the model using the O_2 uptake curve, including respiration timings and speed, were measured directly for the present study, values for the parameters BMR, maximum E_{O2} per breath, and MR_L were estimates based upon available information. The degree of deviation of these parameter values from their true values was uncertain. Therefore, sensitivity analyses were executed to assess how and to what extend variation of these parameter values would affect the conclusions of the study.

2.2.9.1. Sensitivity rates for metabolic costs of speeds

Because the internal power needed for the killer whales in this study to overcome drag could only be approximated, the slope of MR_L vs. speed^3 (k) according to Fig. 2.4 potentially deviated

from its true value. Williams *et al.* (1993) found a MR of 25 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ for a bottlenose dolphin swimming approximately 3 $m \cdot s^{-1}$. According to this MR an adult male killer whale of 3,913 kg and an adult female killer whale of 2,800 kg, accounting for an order of magnitude difference in the mass of dolphins, would use 0.7153 $LO_2 \cdot s^{-1}$ and 0.5565 $LO_2 \cdot s^{-1}$, respectively, for the same swimming speed. According to Eq. 10 this meant that the slope of MR_L vs. speed^3 would be 0.0795 and 0.06183 for males and females, respectively, as tested as the highest value for the sensitivity analyses. Because of lack of published MR estimates for cetaceans below the used values, the lower limit of the sensitivity analyses was set at half the value used, 0.0031 and 0.0025 for male and female killer whales, respectively.

2.2.9.2. Sensitivity rates for basal metabolic costs

BMR values as used in the O_2 model for both sexes were derived from Kriete (1995). However, Kriete (1995) only calculated BMR's from measurements on one adult male and one adult female. Kasting *et al.* (1989) found an equation to calculate BMR for killer whales and beluga whales that approximated 0.88 mass $^{1.06}$. According to this equation an adult male killer whale of 3,913 kg and an adult female killer whale of 2,800 kg would have a BMR of 0.2814 and 0.1974 $LO_2 \cdot s^{-1}$, respectively. These values were used as the upper limits for the sensitivity analysis of this parameter. Recently, Worthy *et al.* (2013) measured a BMR of 196.6 $MJ \cdot d^{-1}$ for a resting adult male killer whale (5,318 kg) using open flow respirometry, which would be equal to a BMR of 0.090 and 0.070 $LO_2 \cdot s^{-1}$ for the adult male and female killer whales, respectively, in the present study, which were set as the lower limits of the sensitivity analysis.

2.2.9.3. Sensitivity rates for maximum O_2 uptake per breath

Both the upper and lower limits for the sensitivity analyses of E_{O2} values for both sexes were derived from Kriete (1995), who measured a maximum V_T for both male and female of 258.5 L and 149.0 L, respectively. For setting the upper limits of the sensitivity analysis these values were multiplied with the percentage of O_2 in air of 20.95. The same was done for the lower limits shaped by the minimum V_T of 82.0 L and 50.5 L found by Kriete (1995) for the adult male and female killer whale, respectively.

2.2.10. Metabolic cost of transport calculations

Estimated E_{O2} found over the 15 min intervals through the different variations of the O_2 model were multiplied with the conversion factor of 20.1 $kJ \cdot LO_2^{-1}$ to derive estimates in J. These values were divided by the speed measurements over the same 15 min interval to derive metabolic cost of transport COT ($J \cdot m^{-1}$). Finally the COT estimates were divided by the body mass per sex category to calculate the mass-specific COT ($J \cdot kg^{-1} \cdot m^{-1}$). The influence of the different O_2 uptake models modifications on the mass-specific COT was examined.

2.3. Results

15 tag records were available for analysis. Data on one adult female was excluded from analyses due to uncertainties related to calculating the pitch, roll and heading data. Other tag records of one adult male and one adult female were excluded due to short tag deployments (<45 min). Consequently, the total amount of DTAG data analysed during the present study was 57.5 hours, from 12 adult killer whales, including six males and six females. Individual tag records ranged from 1.67 hours to almost 12.5 hours in length. Some whales were tagged simultaneously (Table 2.3).

Four whales were experimentally exposed to sonar (Miller *et al.*, 2011; 2014) which is likely to have affected the speed of these individuals during some periods of their tagging record.

2.3.1. Respiration rates

Respiration rates averaged over 15 min intervals ranged from 1.040 to 2.184 $breaths \cdot min^{-1}$ for all whales together (Table 2.4). The highest respiration rate was observed for one of the males (09_144b), while the lowest rate was detected for one of the females (05_320a) (Table 2.4). The total average breathing rate (± standard deviation) was 1.504 (± 0.2378) $breaths \cdot min^{-1}$, while males exhibited a mean rate which was significantly higher (Wald-test, $P = 0.032$) than the mean females' breathing rate, counting 1.558 (± 0.2265) and 1.393 (± 0.2230) $breaths \cdot min^{-1}$, respectively (Table 2.4).

Table 2.3. Information on all tagged adult killer whales included in analyses during the present study

Whale ID	Sex	# 15 min blocks	Calf presence	Sonar	Comments
05_316a	F	7			
05_320a	F	16	√		Tagged simultaneously in different social groups. 05_320b was a large adult.
05_320b	M	5			
05_321b	F	4			
05_322a	F	11	√		Tagged simultaneously in the same social group 05_322b was a large adult.
05_322b	M	9			
06_313s	F	6	√ (juvenile)		
06_314a	M	6			
06_327s	F	19	√	√	Tagged simultaneously in the same social group
06_327t	M	20		√	
09_144a	M	44		√	Tagged simultaneously in the same social group. 09_144b was a large adult compared to 09_144a
09_144b	M	47		√	

Table 2.4. Average, minimum and maximum breathing rates (breaths·min^{-1} over 15 min intervals) per individual killer whale, sex category and for all whales together.

Whale ID	Mean breathing rate min^{-1} (± SD)	Min breathing rate min^{-1}	Max breathing rate min^{-1}
05_316a	1.548 (± 0.18)	1.309	1.880
05_320a	1.163 (± 0.08)	1.040	1.302
05_321b	1.248 (± 0.02)	1.228	1.278
05_322a	1.279 (± 0.19)	1.076	1.614
06_313s	1.553 (± 0.22)	1.249	1.750
06_327s	1.575 (± 0.09)	1.374	1.747
All females	**1.393 (± 0.22)**	**1.040**	**1.880**
05_320b	1.569 (± 0.12)	1.446	1.746
05_322b	1.608 (± 0.29)	1.132	2.029
06_314a	1.443 (± 0.15)	1.250	1.632
06_327t	1.673 (± 0.15)	1.302	1.914
09_144a	1.535 (± 0.17)	1.319	1.887
09_144b	1.534 (± 0.29)	1.164	2.184
All males	**1.558 (± 0.23)**	**1.132**	**2.184**
Overall	**1.504 (± 0.24)**	**1.040**	**2.184**

2.3.2. Speed from flow noise measurements

For ten of the whales, for which both large body pitch angles and acoustics were available, a unique exponential relationship was found between speed and SPL per individual and per tag period between tag movements (Fig. 2.6, Table 2.6) which was used to calculate speed over the entire tag record. Animals 05_316a, 05_322b and 06_327s exhibited no tag movements during deployment. The tags deployed on males 09_144a and 09_144b both moved ten times. The exponential curve of female 05_316a was the steepest off all curves, meaning that her speed was estimated to be higher than for others for a same SPL value (Fig. 2.6, Table 2.6). Females 05_321b and 06_313s showed a relationship with the mildest slope, meaning that speed increased considerably more moderately with increasing SPL compare to for instance 05_316a. Those variations are likely due to differences in tag location and orientation on the body.

Table 2.5. Average, minimum and maximum speed measurements ($m \cdot s^{-1}$ over 15 min intervals) per individual killer whale, sex category and for all whales together.

	Whale ID	Mean speed (± SD)	Min speed	Max speed
Female	05_316a	1.533 (± 0.5960)	0.786	2.358
	05_321b	1.148 (± 0.1501)	1.013	1.299
	05_322a	1.495 (± 0.6175)	0.724	2.802
	06_313s	1.615 (± 0.2416)	1.276	2.010
	06_327s	2.062 (± 0.5348)	1.006	2.743
	All females	**1.716 (± 0.588)**	**0.724**	**2.802**
Male	05_320b	1.222 (± 0.3668)	0.931	1.795
	05_322b	1.273 (± 0.4519)	0.690	1.909
	06_327t	1.498 (± 0.2770)	1.155	2.036
	09_144a	1.953 (± 0.4758)	0.946	3.637
	09_144b	2.361 (± 0.5560)	1.308	4.050
	All males	**1.955 (± 0.6114)**	**0.690**	**4.050**
	Overall	**1.890 (± 0.6127)**	**0.690**	**4.050**

Table 2.6. Parameter values, regression equations between speed and sound pressure level (SPL) and their fit per individual killer whale and per tag period, in chronological order. Numbers of tag movements, and number of gradual ones, are indicated per whale ID. Tag periods without (enough) large angles are not shown.

	Whale ID	Low-pass (Hz)	Min. pitch (deg.)	Equation speed (tag period)	r^2	RMSE
Female	05_316a (0)	200	45	0.0081 * exp (0.0484 * SPL)	0.871	0.1878
	05_321b (1,0)	200	55	0.0072 * exp (0.0431 * SPL)(1)	0.776	0.1230
			60	0.0091 * exp (0.0404 * SPL)(2)	0.959	0.9594
	05_322a (1,1)	200	45	0.0036 * exp (0.0501 * SPL)(1)	0.580	0.4786
				0.0014 * exp (0.0577 * SPL)(2*)		
			65	0.0009 * exp (0.0612 * SPL)(3)	0.874	0.2868
	06_313s (3,0)	350	55	0.0581 * exp (0.0287 * SPL)(1)	0.828	0.2238
			55	0.0313 * exp (0.3256 * SPL)(3)	0.533	0.3547
	06_327s (0)	200	65	0.0064 * exp (0.0467 * SPL)	0.901	0.2293
Male	05_320b (3,1)	250	45	0.0003 * exp (0.0680 * SPL)(1)	0.536	0.2752
				0.0005 * exp (0.0627 * SPL)(2*)		
			65	0.0011 * exp (0.0563 * SPL)(3)	0.865	0.1901
	05_322b (0)	200	65	0.0026 * exp (0.0506 * SPL)	0.823	0.1926
	06_327t (3,1)	150	45	0.0002 * exp (0.0735 * SPL)(1)	0.822	0.2877
	09_144a	250	65	0.0109 * exp (0.0416 * SPL)(1)	0.815	0.3832
	(10,1)		45	0.0040 * exp (0.0501 * SPL)(2)	0.807	0.4072
	09_144b	200	55	0.0016 * exp (0.0578 * SPL)(1)	0.940	0.2917
	(10.6)		45	0.0051 * exp (0.0469 * SPL)(2)	0.886	0.4018
			65	0.0128 * exp (0.0404 * SPL)(4**)	0.829	0.2381
			65	0.0119 * exp (0.0422 * SPL)(5**)	0.725	0.4917
			45	0.0003 * exp (0.0677 * SPL)(8)	0.658	0.3063

* Gradual tag movement; average correlation between previous and following tag period, not based on any data points

** Gradual tag movement; handled like tag period without gradual movement

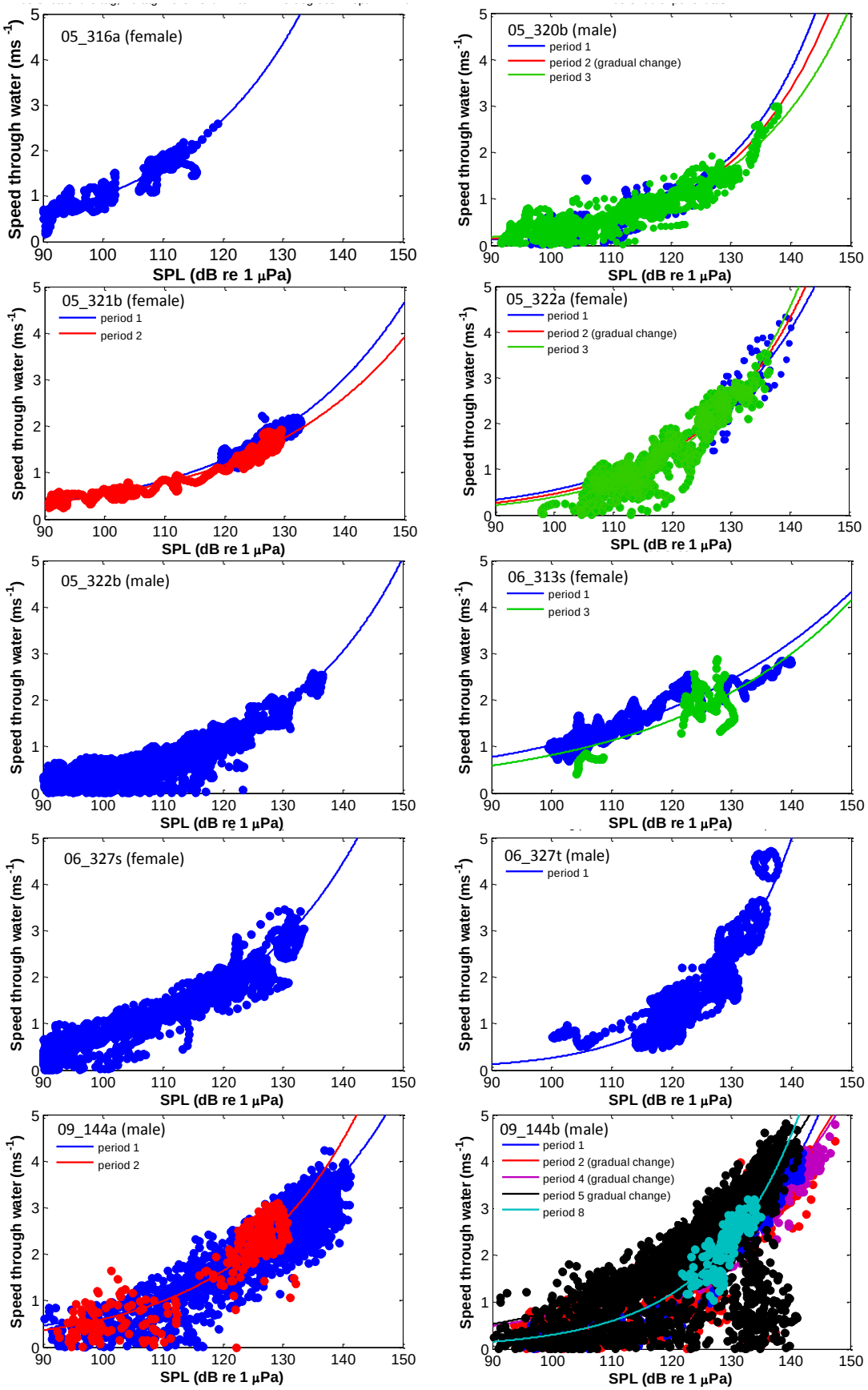

Figure 2.6. Scatter plots of speed through water against sound pressure levels (SPL) per individual killer whale including the fitted exponential model of which information is presented in Table 2.5.

Time-series of the speed measurements, including and excluding outliers, are presented in Appendix 2.1. For all killer whales together a minimum, maximum and average (± standard deviation) speeds over 15 min intervals were 0.69, 4.05, and 1.89 (± 0.613) $m \cdot s^{-1}$, respectively, with both extremes exhibited by males (Table 2.5). The range of speed estimates for females was somewhat smaller, ranging from 0.72 to 2.80 $m \cdot s^{-1}$ on average over 15 min intervals (Table 2.5). The difference between the mean speed over 15 min intervals measured for females (1.72 ± 0.588, Table 2.5) and males (1.96 ± 0.611) was not significant (Wald-test, $P = 0.680$). Male 09_144b displayed an average speed reasonably higher than all other males (Table 2.5).

2.3.3. O_2 use versus activity for different O_2 models

2.3.3.1. The O_2 model using fixed O_2 uptake per breath

A relatively weak correlation existed between breathing rate and speed for females ($r^2 = 0.233$, RMSE = 0.0030), whereas no correlation was apparent for males or all whales together (Table 2.7, Fig. 2.7). There existed no statistical evidence that respiration rate was influenced by sex besides speed (Wald-test, $P = 0.0928$).

Table 2.7. Regression statistics and relationship values between speed and respiration rate per sex category and for all killer whales together.

	# 15 min blocks	r^2	RMSE	Intercept	Slope
Females	47	0.233	0.0030	0.0198	0.0028
Males	125	0.028	0.0038	0.0240	0.0010
All	172	0.071	0.0036	0.0226	0.0016

The E_{O2} estimated through the O_2 model including the fixed O_2 uptake per breath, as used in literature upon to date, showed no correlation with speed over the 15 min intervals for any of the individual whales or categories when setting the intercept at BMR (Table 2.8), meaning that a horizontal line at the average E_{O2} value fitted better than the model. The fitted GEE model with intercept set at BMR lay well below all data points for both categories (see Fig. 2.10) which was no different for the individuals (Table 2.8). When not setting the intercept the model fit increased to an r^2-value of 0.227 for the females, yet did not result in a correlation for the males (Table 2.8). The model showed a moderate fit for half of all individuals,

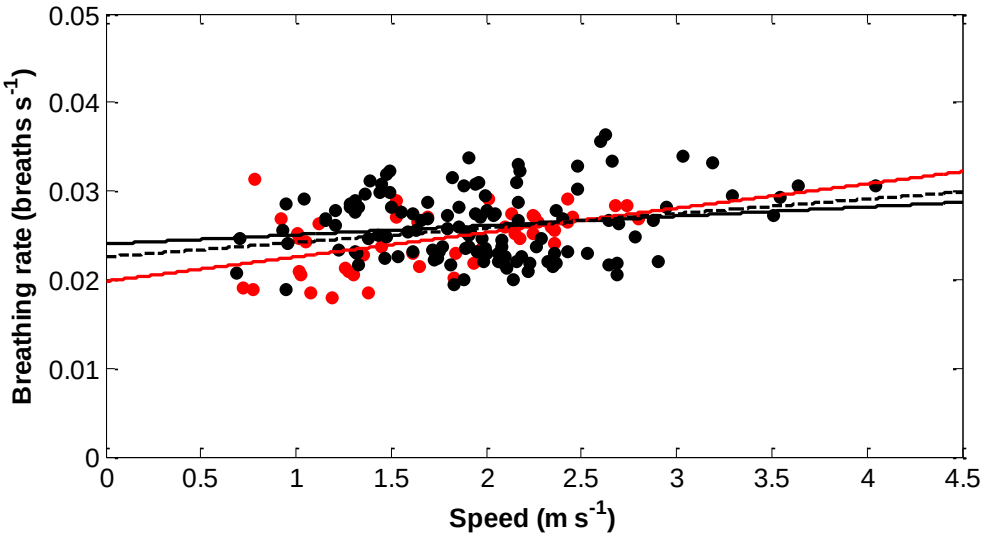

Figure 2.7. Scatter plot of breathing rate averaged over 15 min blocks versus speed averaged over 15 min blocks. Solid lines represent the fitted model for females (red) and males (black). The dashed line represents the fitted model for all individuals together.

Table 2.8. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using a fixed uptake per breath (with and without set intercept at BMR), versus level of activity represented by speed^3 over 15 min intervals for individuals and sex categories.

		Intercept set at BMR			No set intercept			
Female	**# 15 min blocks**	**r^2***	**RMSE**	**Slope**	**r^2***	**RMSE**	**Intercept**	**Slope**
05_316a	7	-46.174	0.2317	0.0010	0.499	0.0239	0.3357	-0.0060
05_321b	4	-175.528	0.0548	0.0968	-0.012	0.0041	0.2454	0.0016
05_322a	11	-19.391	0.1605	0.0043	0.623	0.0218	0.2304	0.0045
06_313s	6	-2.773	0.0789	0.0464	0.2750	0.0346	0.2573	0.0113
06_327s	19	-131.023	0.2069	0.0032	0.438	0.0135	0.2879	0.0023
All females	**63**	-26.313	0.2061	0.0023	0.227	0.0347	0.2728	0.0026
Male								
05_320b	5	-126.019	0.4561	0.0107	0.038	0.0397	0.5706	0.0061
05_322b	9	-14.945	0.4057	0.0335	0.559	0.0675	0.5152	0.0310
06_327t	20	-82.653	0.4973	0.0062	-0.071	0.0563	0.5954	0.0056
09_144a	44	-53.733	0.4559	0.0015	0.075	0.0592	0.5651	0.0015
09_144b	47	-19.924	0.4812	-0.0003	-0.088	0.1097	0.6010	-0.0002
All males	**131**	-27.400	0.4507	0.0034	0.0066	0.0843	0.5655	0.0029

* Negative r^2 shows that the fit of the model is worse than the fit of the mean y-value (null hypothesis).

including four of the females and one of the males (Table 2.8). Slopes of the fitted model without set intercept were comparable of those for the fitted model with the intercept set at BMR for both categories and the majority of the individuals (Table 2.8, see Fig 2.10). Whereas intercepts were considerably higher than BMR values for the model without the set intercept for as well individuals as categories (Table 2.8). The BMR value of the categories according to the model without the intercept set at BMR was estimated to be 3.7 and 5.4 times higher than the BMR values used for females and males, respectively (Table 2.8, see Fig. 2.10).

Applying the O_2 model including the fixed E_{O2} per breath as employed by Williams and Noren (2009), resulted in an unrestrained and unrealistically high O_2 store for all individuals, well exceeding their potential TBO, meaning there was no balance between the O_2 use and uptake over the tag period (Fig. 2.8, Appendix 2.2).

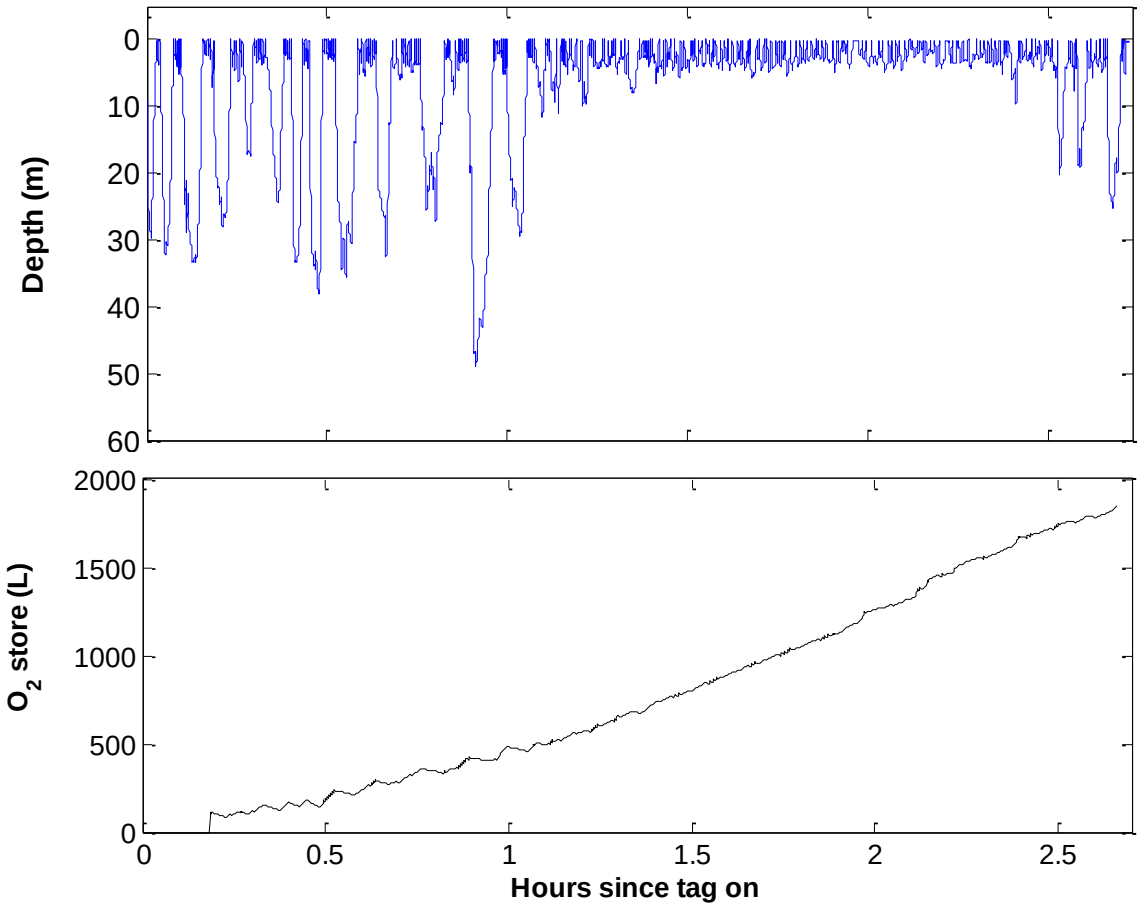

Figure 2.8. Example of a time series plot of female 05_316a of the dive profile (top) and O_2 store estimated over the entire tag record through the O_2 model with fixed O_2 uptake per breath (bottom) as used in existing literature, including Kriete (1995) and Williams and Noren (2009) (bottom).

2.3.3.2. The O_2 model using an optimized fixed O_2 uptake

All optimized fixed E_{O2} per breath were considerably lower than the values used by Williams and Noren (2009), with significantly different (Wald-test, $P = 0.002 * 10^{-13}$) average optimized values of 4.56 and 6.09 LO_2 per breath for adult female and male killer whales, respectively (Table 2.9). The optimized values for females and males ranged between 3.77 and 5.94, and 4.76 and 8.92 LO_2 per breath, respectively (Table 2.9). Only for one female and one male the r^2-value of the fitted O_2 model including the optimized O_2 uptake per breath was positive, showing a moderate fit to the data (Table 2.9). Concerning the model fitted per category, the variation in E_{O2} showed no relationship with speed^3, as for using the non-optimized fixed uptake (Table 2.9, see Fig. 2.10). The slope of one male was negative. The slopes for the poor fitting models per category showed a similar slope (Table 2.9, see Fig. 2.10).

Table 2.9. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using an optimized fixed uptake per breath (with intercept set at BMR), versus level of activity represented by speed^3 over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized fixed E_{O2} (L)	r^{2*}	RMSE	Slope
Female	05_316a	7	4.19	-7.440	0.0345	0.0007
	05_321b	4	3.99	-4.696	0.0033	0.0055
	05_322a	11	5.94	-5.139	0.0440	0.0022
	06_313s	6	3.77	0.201	0.0115	0.0049
	06_327s	19	4.89	-31.440	0.0422	0.0013
Average fixed E_{O2} and stats			**4.56**	**-4.371**	**0.0350**	**0.0009**
Male	05_320b	5	5.04	-5.325	0.0230	0.0026
	05_322b	9	4.87	0.531	0.0152	0.0084
	06_327t	20	4.76	-3.816	0.0255	0.0014
	09_144a	44	6.88	-12.276	0.0693	0.0005
	09_144b	47	8.92	-8.803	0.1317	-0.0001
Average fixed E_{O2} and stats			**6.09**	**-3.675**	**0.0499**	**0.0010**

* Negative r^2 shows that the fit of the model is worse than the fit of the mean y-value (null hypothesis).

When not setting the intercept of the model including the optimized O_2 uptake per breath at BMR the fit increased considerably for all individuals. Still, no relationship was revealed between E_{O2} and speed^3 for one females, two males and males as category. For female

05_316a, showing a moderate fit, a negative relationship was discovered (Table 2.10). The fits of the model per category were worse than per individual, except for one female and two males (Table 2.10). The variance of the model fitted per category was larger than model fit per individual, except for male 09_144b (Table 2.10), enlightening that the variation was increasing by grouping individuals. This was also indicated by the variation in slopes (Table 2.10). The slope of males and females was quite similar (Table 2.10, see Fig. 2.10). All intercepts were higher than the BMR value used in the O_2 model (Table 2.10). The intercept for both categories were approximately 1.5 times higher than the used BMR values.

Table 2.10. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using an optimized fixed uptake per breath (without set intercept), versus level of activity represented by speed^3 over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized fixed E_{O2} (L)	r^{2*}	RMSE	Slope	Intercept
Female	05_316a	7	4.19	0.499	0.0084	-0.0021	0.1182
	05_321b	4	3.99	-0.012	0.0014	0.0005	0.0823
	05_322a	11	5.94	0.623	0.0109	0.0023	0.1150
	06_313s	6	3.77	0.275	0.0110	0.0036	0.0815
	06_327s	19	4.89	0.438	0.0056	0.0010	0.1183
Average fixed E_{O2} and stats			**4.56**	**0.227**	**0.0133**	**0.0010**	**0.1045**
Male	05_320b	5	5.04	0.038	0.0090	0.0014	0.1290
	05_322b	9	4.87	0.559	0.0147	0.0068	0.1125
	06_327t	20	4.76	-0.071	0.0120	0.0012	0.1271
	09_144a	44	6.88	0.075	0.0183	0.0005	0.1743
	09_144b	47	8.92	-0.088	0.0439	-0.0001	0.2404
Average fixed E_{O2} and stats			**6.09**	**0.007**	**0.0230**	**0.0008**	**0.1544**

* Negative r^2 shows that the fit of the model is worse than the fit of the mean y-value (null hypothesis).

Though by definition, the accumulated E_{O2} over each time-series was set to be equal to the accumulated V_{O2}, O_2 stores estimated through the O_2 model with the fixed E_{O2} per breath optimized per individual resulted in an unrealistically temporary excess of the estimated TBO and/or a negative O_2 store for all individuals (Fig. 2.9, Appendix 2.2).

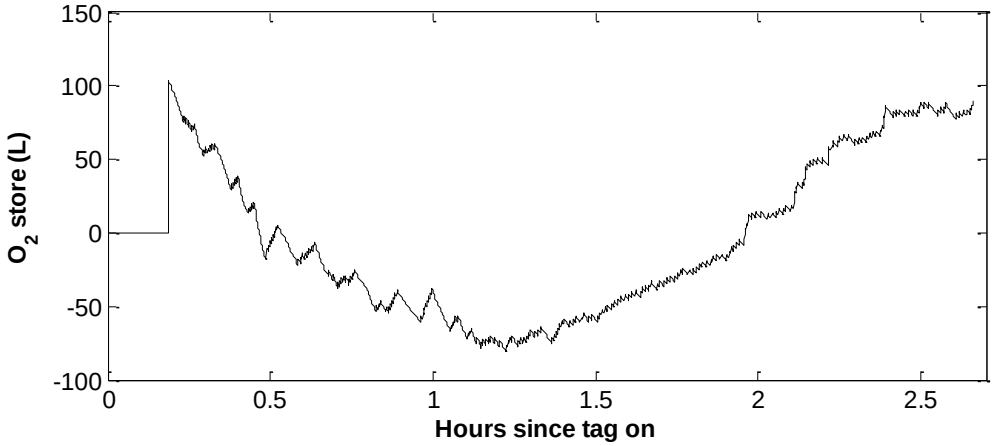

Figure 2.9. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 2.9) estimated over the entire tag recored through the O_2 model including an optimized fixed O_2 uptake per breath.

2.3.3.3. The O_2 model including a set O_2 uptake curve

Including a fluctuating O_2 uptake to the model through inclusion of a set O_2 uptake curve data of all individuals led to a strong fit between E_{O2} and speed^3 over 15 min intervals, when the intercept was set at BMR (Table 2.11, Fig.2.10). In the case of the females the overall r^2 value was lower than all individuals, whereas for the males this value was higher than all individuals (Table 2.11). The fact that the r^2 value for all males or females together did not fall within the r^2 value ranges found for the individuals could be explained by the slope differences between the individuals which are added to the GEEGLM model as random factors (Table 2.11). However, the range in slopes within both categories was rather similar ranging from 0.0054 to 0.0084 and 0.0066 to 0.0112 for females and males, respectively, as was the slope of the model fitted to both categories (Table 2.11, Fig.2.10). Of the females 05_322a had the steepest slope as relation between E_{O2} and speed^3. The data point representing the highest speed and E_{O2}, showing the greatest deviation, belonged to this particular female.

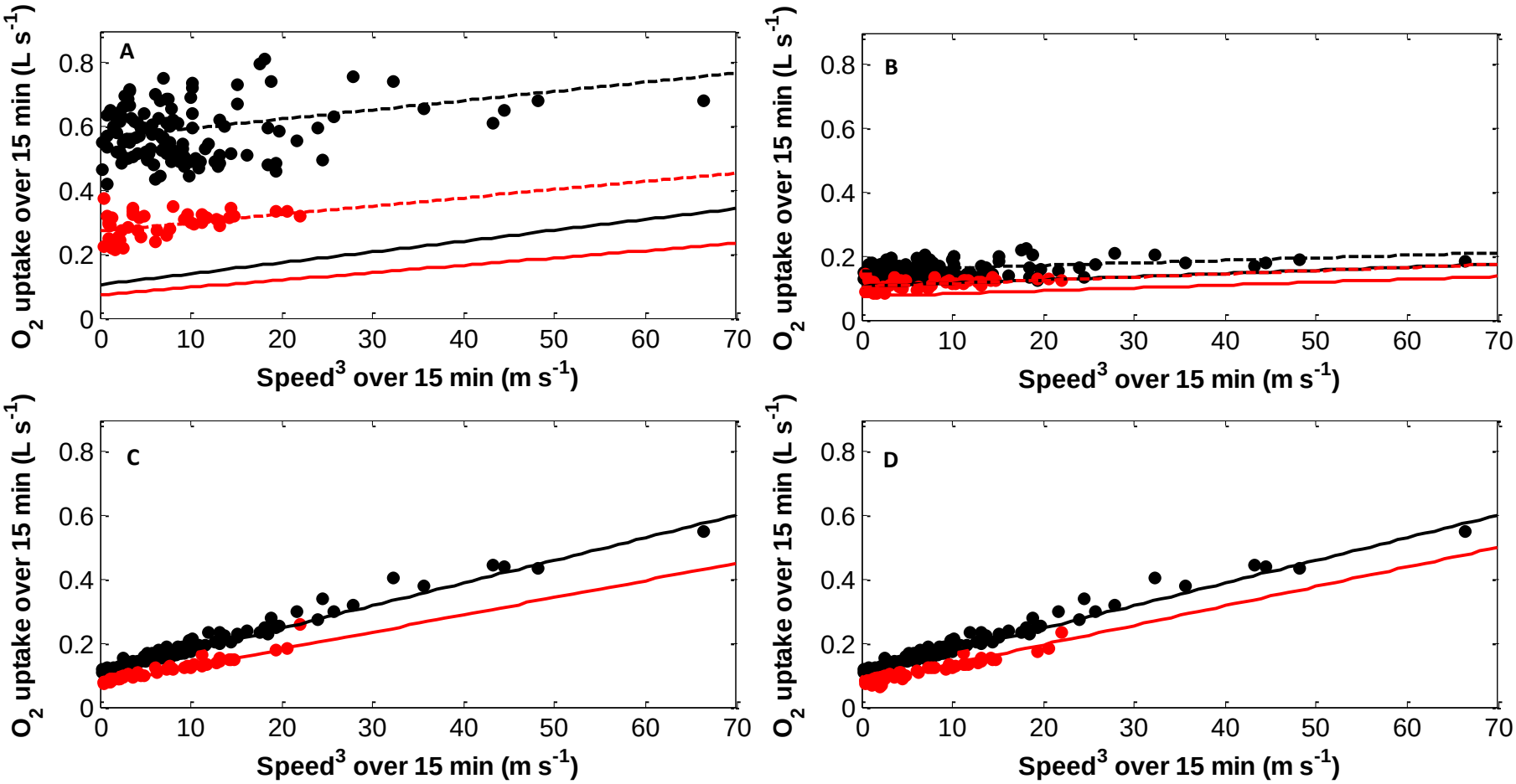

Figure 2.10. O_2 uptake versus speed^3, both average over 15 min intervals, estimated through the O_2 model with fixed O_2 uptake(**A**), optimized fixed O_2 uptake (**B**), fluctuating O_2 uptake according to a set O_2 uptake curve (**C**), and fluctuating O_2 uptake according to an optimized O_2 uptake curve (**D**) for males (black) and females (red). Solid lines represent the O_2 model fitted with intercept set at BMR, dashed lines represent the O_2 model without set intercept.

Table 2.11. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using a set O_2 uptake curve (with intercept set at BMR), versus level of activity represented by speed^3 over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	r^2	RMSE	Slope
Female	05_316a	7	0.990	0.0027	0.0064
	05_321b	4	0.952	0.0010	0.0071
	05_322a	11	0.997	0.0028	0.0084
	06_313s	6	0.976	0.0015	0.0057
	06_327s	19	0.981	0.0038	0.0054
	All females	**63**	0.899	0.0112	0.0063
Male	05_320b	5	0.784	0.0095	0.0112
	05_322b	9	0.908	0.0053	0.0085
	06_327t	20	0.912	0.0051	0.0074
	09_144a	44	0.938	0.0143	0.0066
	09_144b	47	0.907	0.0262	0.0069
	All males	**131**	0.954	0.0161	0.0071

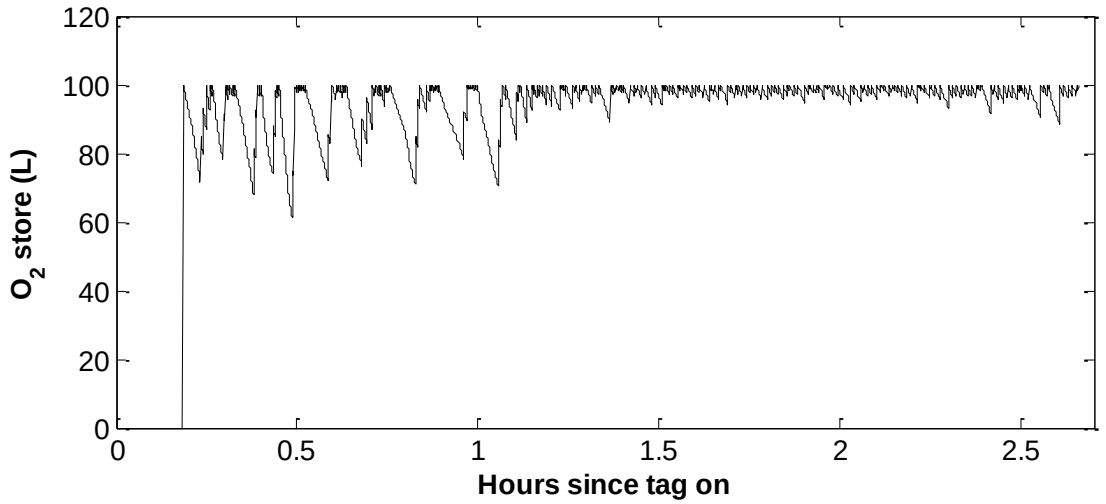

Figure 2.11. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 2.9) estimated over the entire tag recored through the O_2 model including a set O_2 uptake curve.

E_{O2} and V_{O2} were more balanced for all individuals when applying an E_{O2} as a function of O_2 store, without exceding the estimated TBO capacity: when the animals took many breaths in a row after a longer dive the first couple of breaths were sufficient, however as the O_2 store became more saturated, E_{O2} per breath became less efficient (Fig. 2.11). The O_2 store was for none of the whales depleted completely (Appendix 2.2).

2.3.3.4. The O_2 model including an optimized O_2 uptake curve

The optimized exponent for the density dependent curve applied to the O_2 model showed a larger range for males than females, ranging from 5.37 to 11.66 and 1.66 to 5.49, respectively (Table 2.12, Fig. 2.12). However, for one of the females, two of the males, males as category and for all whales together there existed a horizontal asymptote when plotting the r^2 values against the exponent values: the r^2 kept increasing with increasing exponent value. The higher the exponent, the more the curve approached the shape of the set uptake curve (Fig. 2.12) and so in these cases the O_2 model including the non-optimized set uptake curve fitted the data better than a density dependent curve. Still, to make a consistent approximation of the optimized exponent the value was selected which led to an r^2 that was 0.5 % of the total r^2 variance under the highest r^2 value at the horizontal asymptote; increase of the exponent value from hereon only caused an insignificant increase in r^2.

The relationship between speed^3 and estimated E_{O2} over the 15 min intervals was strong for all individuals, categories and for all killer whales together. Yet, model fit was lower for females as a category compared to individual females. Also, the optimized O_2 uptake curve for all females together was physiologically unrealistic: it was expected that uptake during very low O_2 store would be maximal and its decrease increased gradually with higher O_2 store (see Fig. 2.13). Fig. 2.10 revealed that observations belonging to female 05_322a, holding one of the higher model slopes, laid relatively high above the fitted model (Table 2.12). Excluding this individual from category the alternate optimized exponent, of 6.06 (r^2 = 0.977, RMSE = 0.0044, slope = 0.0054), became realistic (see Fig. 2.13 and Fig.2.14). The optimized curve exponent of the males was very similar to the one for all whales together. Still, for males as category and all whales together the O_2 uptake was better predicted by the set uptake curve, rather than the optimized one. Applying the curve exponent optimized for all whales together per sex categories also resulted in strong correlation between estimated E_{O2} and level of activity over 15 min intervals (Table 2.12).

Table 2.12. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using an optimized O_2 uptake curve (with intercept set at BMR), versus level of activity represented by speed^3 over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized exponent	r^2	RMSE	Slope
Female	05_316a	7	3.08	0.996	0.0018	0.0064
	05_321b	4	1.66	0.983	0.0005	0.0073
	05_322a	11	(5.49)*	(0.997)	(0.0030)	(0.0085)
	06_313s	6	3.62	0.982	0.0014	0.0057
	06_327s	19	4.42	0.983	0.0036	0.0053
	All females	**63**	0.20	0.903	0.0108	0.0061
Male	05_320b	5	8.18	0.783	0.0095	0.0112
	05_322b	9	(11.66)*	(0.907)	(0.0053)	(0.0085)
	06_327t	20	(5.37)*	(0.911)	(0.0052)	(0.0074)
	09_144a	44	6.94	0.971	0.0097	0.0074
	09_144b	47	6.69	0.941	0.0210	0.0076
	All males	**131**	(14.51)*	(0.953)	(0.0162)	(0.0071)
	Overall	**194**	(14.30)*	(0.944)	(0.0162)	(0.0070)
	Females			(0.894)	(0.0115)	(0.0064)
	Males			(0.953)	(0.0162)	(0.0071)

* No automatic optimization possible due to the presence of asymptote, see text for further details.

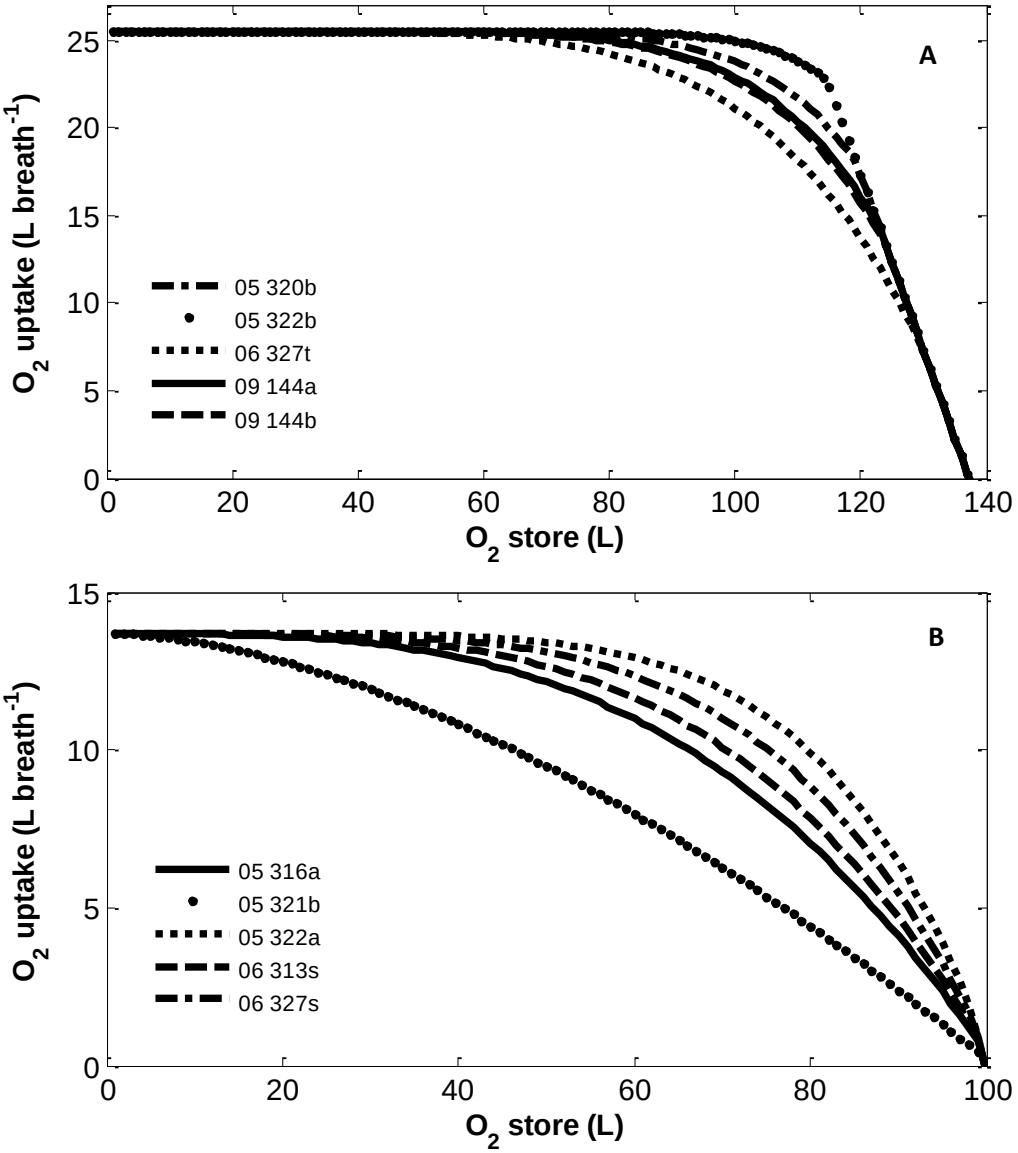

Figure 2.12. The O_2 uptake per breath as a function of the O_2 store at the time of each breath taking the form of a density dependent curve, shaped by the exponent optimized per individual male (A) and female (B).

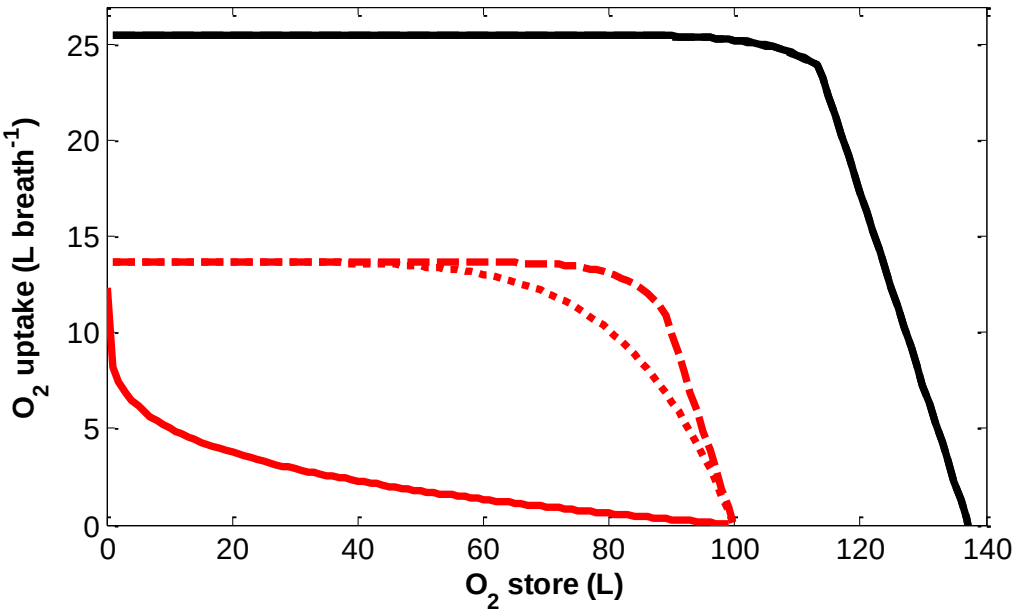

Figure 2.13. The O_2 uptake as a function of the O_2 store at the time of each breath taking the form of a density dependent curve, shaped by the exponent optimized for males (black solid line), females (red solid line), and all killer whales together applied to males (black dashed line) and females (red dashed line). The red dotted line represents the optimized function for females excluding female 05_322a.

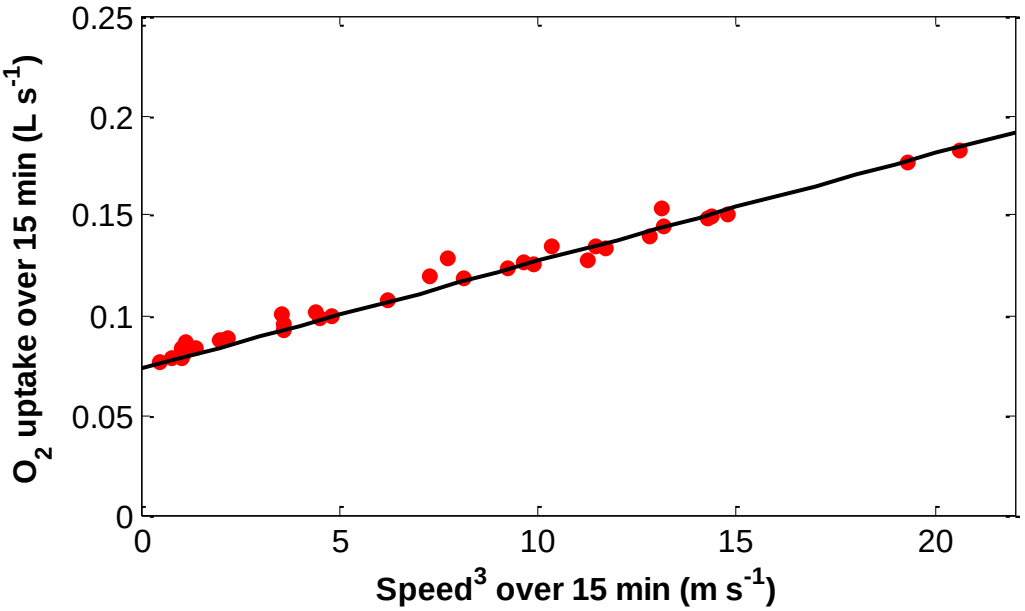

Figure 2.14. Scatter plot of E_{O2} averaged over 15 min blocks estimated, through the O_2 model including an optimized O_2 uptake curve, versus speed^3 averaged over 15 min blocks and the fitted model for females excluding female 05_322a.

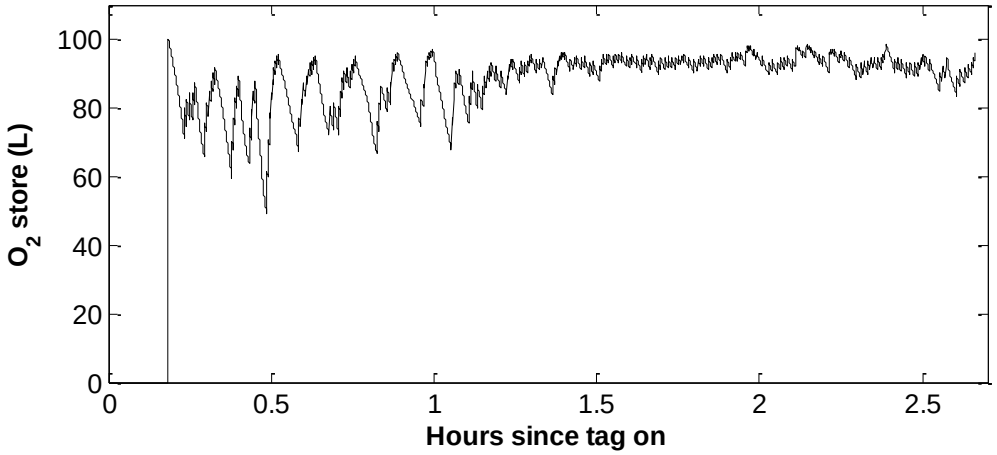

Figure 2.15. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 2.9) estimated over the entire tag recored through the O_2 model including its optimized O_2 uptake curve.

The O_2 store estimated with the O_2 model including the optimized O_2 uptake curve per individual was more balanced than fixed uptake models, without exceeding the estimated TBO capacity (Fig. 2.15).

In summary, these results showed that the O_2 model applied with a fixed E_{O2} per breath led to unrealistically high O_2 stores and to a poor match between E_{O2} and activity over 15 min periods. However, by including a fluctuating E_{O2}, according to the O_2 store at the time of each breath, O_2 stores were constrained to realistic levels. Also, the correlation strength between E_{O2} and activity level increased considerably resulting in a more realistic balance between E_{O2} and O_2 use according to level of activity over 15 min periods.

2.3.4. Sensitivity Analyses

2.3.4.1. Metabolic Rates

Due to the large range in possible energetic parameters derived from literature, the corresponding model statistics showed great variation (see Table 2.14). By increasing the values used for slope of MR_L vs. speed^3 by 13.1 and 12.6 times for males and females, respectively, the model fit became worse than a horizontal line at mean E_{O2} for both sexes (see Table 2.14, see Fig. 2.19). By decreasing the slope of MR_L vs. speed^3 for females by 25 %, the r^2

increased with 4.1 % (see Table 2.14). By halving the slope of MR_L vs. speed^3 for females the r^2 increased with 4.2 % (see Table 2.14). Decreasing the slope of MR_L vs. speed^3 for males by either 25 or 50 % the model fit stayed the same. Yet RMSE did decrease (see Table 2.14). For both sexes model slope decreased for the two lower slopes of MR_L vs. speed^3 (see Table 2.14, see Fig. 2.19).

When applying the two highest slopes of MR_L vs. speed^3 as sensitivity analysis to the model with the set O_2 uptake curve, the whales would replenish O_2 store maximally with every breath (Fig. 2.16), indicating that according to the model it was impossible for them to replenish their O_2 store. The number of breaths with maximum E_{O2} decreased with decreasing slope of MR_L vs. speed^3, and so the number of breaths with lower E_{O2} increased (Fig. 2.16). E_{O2} of male 09_144b would either be maximum or below 12 L, with a peak of occurrence of breaths between 2 and 5 L (Fig. 2.16).

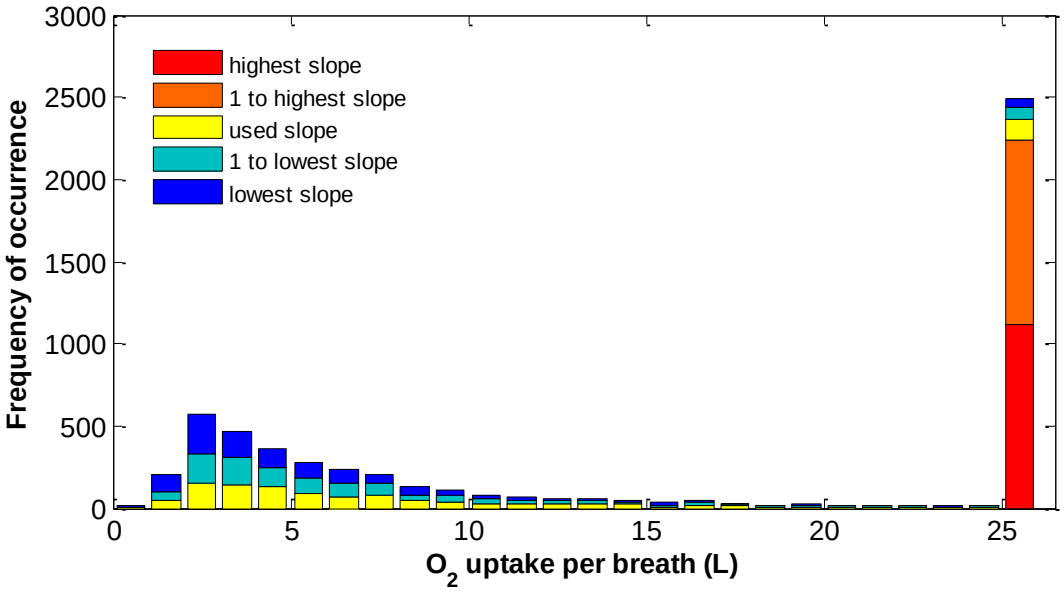

Figure 2.16. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for the slope of MR_L vs. speed^3 (k) incorporated into the O_2 model with a set O_2 uptake curve.

Also the slope of MR_L vs. speed^3 was optimized according to the model with the fixed E_{O2}, as used by Williams and Noren (2009), and the optimized fixed E_{O2} through the following equation:

$$k = (\Sigma E_{O2} - (BMR * t)) / \Sigma (v^3) \quad (16)$$

Where *k* is the slope of MR_L vs. speed^3, ΣE_{O2} is the complete O_2 uptake over the entire tag record calculated by multiplying the number of breaths with the fixed E_{O2}, t is the total length of the tag record in seconds, and *v* is the speed in $m \cdot s^{-1}$ estimated for every second of the tag record. To establish a balance between E_{O2} and V_{O2} over entire tag records according to the fixed E_{O2} uptake per breath, averaged slope of MR_L vs. speed^3 values of 0.067 and 0.103 for females and males, respectively, were found. These values were 13.6 and 16.8 times larger, respectively, than the slope of MR_L vs. speed^3 used in the present study (Fig. 2.4). According to the optimized fixed E_{O2} the slope values became 0.013 and 0.012 for females and males, being 2.1 and 1.7 times larger, respectively, than the values used. By applying values for slope of MR_L vs. speed^3 found according to the fixed E_{O2} to the O_2 model with O_2 uptake curve, there appeared to be no correlation between E_{O2} and activity level (Table 2.13). By applying the values for slope of MR_L vs. speed^3 found according to the optimized fixed E_{O2} to the O_2 model with O_2 uptake curve, the correlation between E_{O2} and V_{O2} increased considerably (Table 2.13). Understandably, the correlation slope for both categories was higher than when applying the original slope of MR_L vs. speed^3 to the O_2 model with uptake curve.

Table 2.13. Regression statistics per category for O_2 uptake versus speed^3 over 15 min when applying the optimized slope of MR_L vs. speed^3 found according to fixed O_2 uptake and optimized fixed O_2 uptake. O_2 uptake modelled was estimated over 15 min intervals according to the O_2 model with a set O_2 uptake curve. Intercept was set at BMR.

	Slope of MR_L vs. speed^3 (*k*) (fixed E_{O2})				**Slope of MR_L vs. speed^3 (*k*) (opt. fixed E_{O2})**			
	k	***r*^{2}**	**RMSE**	**Slope**	***k***	***r*^{2}**	**RMSE**	**Slope**
Females	0.067	-11.053	0.2147	0.0057	0.014	0.463	0.0608	0.0129
Males	0.103	-11.187	0.4842	0.0044	0.011	0.847	0.0512	0.0117

2.3.4.2. Basal Metabolic Rates

Though when implementing different BMR the intercept of the model changed, the slope, and so the relationship between E_{O2} and speed^3 did not change critically (Table 2.14, see Fig. 2.19). A BMR of 2.7 times the used value made the r^2 decrease by 4.7 and 13.2 % for males and females, respectively, while a BMR of 1.17 and 1.04 times smaller for males and females, respectively, yielded same r^2 values as for used BMR for both males and females (Table 2.14). Model slope increased by 1.6 % for the highest BMR tested for females, yet for the males the slope decreased by 1.4 % (Table 2.14). Still, applying any of the BMR values to the O_2 model

Table 2.14. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using a set O_2 uptake curve (with intercept set at BMR), versus level of activity represented by speed^3 over 15 min intervals, according to different slopes of MR_L vs. speed^3 (*k*), basal metabolic rates, and maximum O_2 uptake per breath per sex category.

Males	**Slope of MR_L vs. speed^3**	r^2	**RMSE**	**Slope**
(125 blocks)	**0.080**	-7.620	0.4669	0.0046
	0.043	-3.840	0.4181	0.0049
	0.005	0.955	0.0130	0.0058
	0.003	0.954	0.0079	0.0035
	Basal metabolic rate	r^2	**RMSE**	**Slope**
	0.281	0.909	0.0228	0.0070
	0.193	0.945	0.0177	0.0071
	0.098	0.954	0.0161	0.0071
	0.090	0.954	0.0160	0.0071
	Max. O_2 uptake per breath	r^2	**RMSE**	**Slope**
	54.16	0.957	0.0155	0.0071
	39.84	0.957	0.0156	0.0071
	22.29	0.949	0.0169	0.0071
	19.06	0.923	0.0206	0.0069
Females	**Slope of MR_L vs. speed^3**	r^2	**RMSE**	**Slope**
(47 blocks)	**0.062**	-9.534	0.2114	0.0060
	0.033	-3.523	0.1758	0.0081
	0.004	0.936	0.0067	0.0049
	0.002	0.937	0.0033	0.0024
	Basal metabolic rate	r^2	**RMSE**	**Slope**
	0.197	0.780	0.0174	0.0064
	0.135	0.793	0.0182	0.0071
	0.072	0.896	0.0113	0.0064
	0.070	0.899	0.0111	0.0064
	Max. O_2 uptake per breath	r^2	**RMSE**	**Slope**
	31.22	0.946	0.0075	0.0059
	22.45	0.945	0.0076	0.0059
	12.06	0.833	0.0157	0.0068
	10.43	0.806	0.0172	0.0069

with a set O_2 uptake curve yielded a better model fit than the O_2 model with the optimized fixed E_{O2}.

When applying the two higher BMR values to the model with O_2 uptake curve for male 09_144b, more breaths would have been taken with maximum E_{O2} and breaths with other E_{O2} would be more equally distributed (Fig. 2.17). With decreasing BMR the frequency of maximum E_{O2} per breath decreased and a peak in occurrence appeared for E_{O2} between 2 and 6 L per breath (Fig. 2.17).

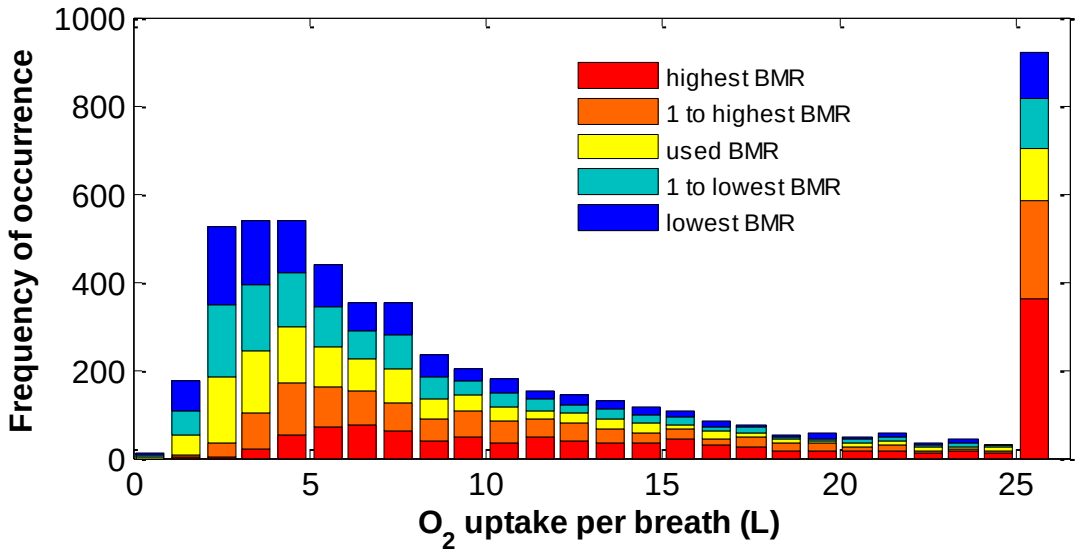

Figure 2.17. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for basal metabolic rate (BMR) incorporated into the O_2 model with a set O_2 uptake curve.

Also the BMR was optimized according to a balance between E_{O2} and V_{O2} using the model with the fixed E_{O2}, as used by Kriete (1995) and Williams and Noren (2009), through the following equation:

$$BMR = (\Sigma E_{O2} - \Sigma (v^3 * k)) / t \quad (17)$$

Average optimized BMR were found counting 0.3525 and 0.6288 for females and males, being 4.8 and 6.0 times bigger, respectively, than the BMR used, yet revealed no correlation between E_{O2} and activity level over 15 min periods when applied to the O_2 model with a set O_2 uptake curve (Table 2.15). The averaged optimized BMR found by balancing E_{O2} and V_{O2}

according to the O_2 model with optimized fixed E_{O2} counted 0.1121 and 0.1273 for respectively females and males (Table 2.15). These calculated BMR values raised correlation strength between E_{O2} and speed^3 as strong as found when using the original values (Table 2.15). This was not unexpected as these values were only 1.5 and 1.2 times larger than the values originally used for females and males, respectively.

Table 2.15. Regression statistics per category for O_2 uptake versus speed^3 for applying the optimized BMR values found according to fixed O_2 uptake and optimized fixed O_2 uptake. O_2 uptake modelled was estimated over 15 min intervals according to the O_2 model with a set O_2 uptake curve. Intercept was set at BMR.

	BMR optimized through fixed E_{O2}				**BMR optimized through opt. fixed E_{O2}**			
	BMR	**r^2**	**RMSE**	**Slope**	**BMR**	**r^2**	**RMSE**	**Slope**
Females	0.3525	-0.233	0.0503	0.0017	0.1121	0.829	0.0159	0.0069
Males	0.6288	0.094	0.0814	0.0035	0.1273	0.952	0.0164	0.0071

2.3.4.3. Maximum O_2 Uptake per Breath

The higher the maximum E_{O2}, the tighter became the correlation between E_{O2} and speed^3 over the 15 min intervals (Table2.14). Especially for the females the r^2 value increased significantly.

For a maximum E_{O2} for females of 2.3 times the used value, the r^2 increased by 5.2 %, whereas an increase of 2.1 times of the used maximum E_{O2} for the males resulted in an increase of the r^2 by only 0.3 % (Table 2.14, see Fig. 2.19). Maximum E_{O2} of 1.3 times smaller than the used value resulted in a decrease of 10.3 and 3.2 % of the r^2 for females and males, respectively. Model slope for females decreased with 6.3 % for both higher values applied and increased with 7.9 and 9.5 % for the one-to-lowest and lowest maximum E_{O2}, respectively (Table 2.14). Model slope for males only decreased by 2.8 % for the lowest maximum E_{O2}, yet stayed the same for the other tested values (Table 2.14, see Fig. 2.19).

The higher the maximum possible E_{O2}, the less frequent this maximum amount would be taken up per breath (Fig. 2.18). The frequency distribution of E_{O2} amounts per breath would be the same for all maximum E_{O2} values for under the lowest maximum E_{O2} value applied (Fig. 2.18).

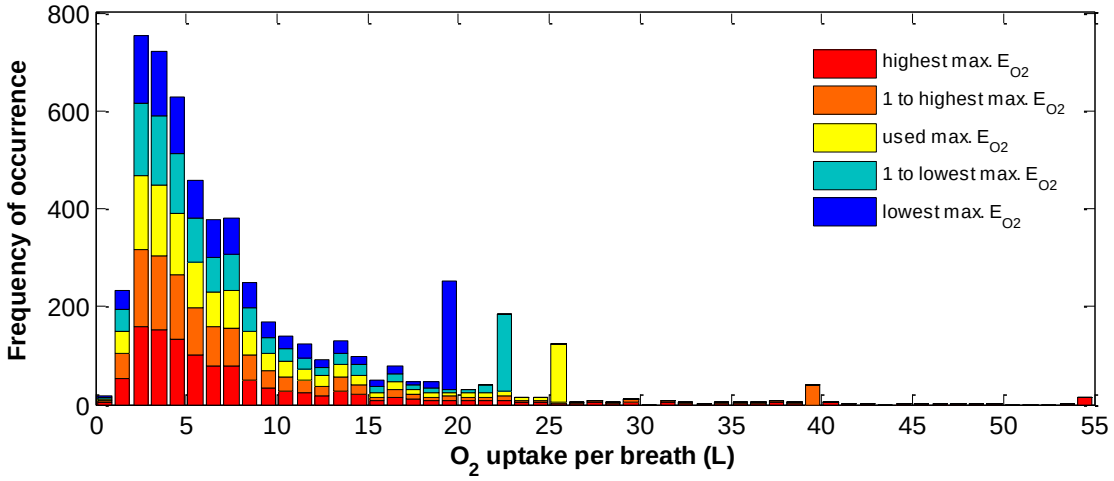

Figure 2.18. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for maximum possible O_2 uptake per breath incorporated into the O_2 model with a set O_2 uptake curve.

In summary, the sensitivity analyses results showed that the O_2 model was especially sensitive to change of slope of MR_L vs. speed^3, in particular when these were higher than the values used, regarding both slope and correlation. Female data were somewhat more sensitive to parameter value changes. Still, even though parameter values were uncertain in the O_2 model including the O_2 uptake curve these results supported the earlier observation that allowing E_{O2} to fluctuate according to the O_2 store at the time of each breath E_{O2} estimates were in balance with the V_{O2} and so more realistic than assuming a fixed E_{O2} per breath.

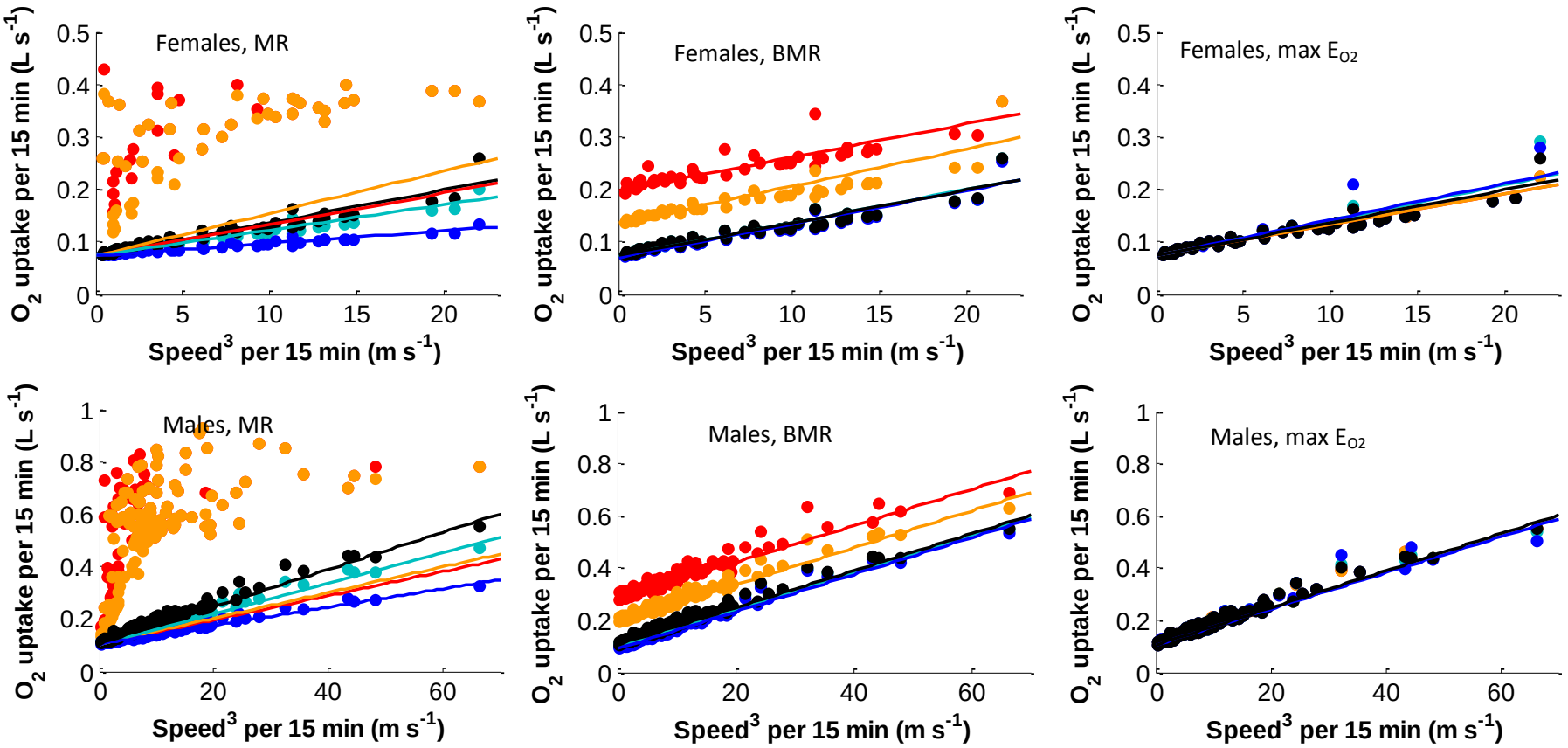

Figure 2.19. O_2 uptake per 15 min intervals as estimated by the O_2 model including a set O_2 uptake curve versus speed^3 per sex category, with different values for the slope of MR_L vs. speed^3 (*k*) (MR), basal metabolic rate (BMR) and maximum O_2 uptake per breath E_{O2}) implemented for sensitivity analyses of the model, where red represents highest, orange one to highest, aqua one to lowest, and blue lowest values. The original model outcomes are coloured black.

2.3.5. Metabolic cost of transport

Correlation between E_{O2} and speed^3 over 15 min intervals, estimated with either the model including fixed or optimized fixed E_{O2}, was strongest by fitting a simple linear regression model and r^2 values were the same per category for both implemented uptakes with the r^2 for the males being extremely low (Table 2.16). However, residual error and overall estimated E_{O2} were lower using the optimized fixed E_{O2} (Table 2.16, Fig. 2.20). The relationship between E_{O2} estimated by fluctuating uptake and linear speed was best represented by a non-linear increasing model, which showed a considerably good fit for both males and females (Table 2.16, Fig. 2.20).

AIC's and r^2 values indicated that COT for female and male killer whales, estimated by the O_2 model using either fixed or optimized fixed E_{O2}, was somewhat better predicted from speed by a power function than a polynomial function (Table 2.16). Moreover, COT kept declining with increasing speed for both genders, and so it was self-evident to fit a power function (Fig. 2.21). In contrast, COT estimated through the O_2 model with fluctuating E_{O2} revealed a broad yet distinct minimum at a speed between 1.7 and 2.2 $m \cdot s^{-1}$ for females and between 1.9 and 2.4 $m \cdot s^{-1}$ for males (Fig. 2.21). Minimum COT estimated with the model including the fixed E_{O2} was the highest (app. 1.0 $J \cdot kg^{-1} \cdot m^{-1}$ for both sexes), while minimum COT estimated by the optimized fixed E_{O2} was the lowest (Fig. 2.21). Minimum COT estimated through the fixed E_{O2} and the fluctuating E_{O2} was similar for both sexes, yet males had a somewhat higher optimum speed (Fig. 2.21). The approximated minimum COT speed was the lowest for COT estimated through fluctuating E_{O2} for both sexes (Table 2.16, Fig. 2.21). Estimated minimum mass-specific COT according to fluctuating E_{O2} was 0.44 and 0.42 $J \cdot kg^{-1} \cdot m^{-1}$ for male and female killer whales, respectively.

Table 2.16. Equations and regression statistics for the correlations between O_2 uptake, estimated by the different model alternatives, versus level of activity represented by speed, and corresponding COT versus speed per sex category. O_2 uptake, COT and speed were all average per 15 min intervals.

O_2 model using fixed E_{O2}					
	Equation		**r^2**	**RMSE**	**App. optimal speed ($m \cdot s^{-1}$)**
E_{O2} 15 min					
Females	0.03272 * speed + 0.236		0.233	0.0353	
Males	0.02333 * speed + 0.536		0.028	0.0840	
COT		**AIC**			
Females	$0.547 * speed^2 - 2.627 * speed + 4.078$	5.91	0.791	0.2446	2.2 – 2.7
	$1.962 * speed^{-0.8773}$	0.715	0.805	0.2338	-
Males	$0.425 * speed^2 - 2.658 * speed + 5.096$	3.24	0.847	0.2403	3.0 – 3.5
	$2.861 * speed^{-0.9277}$	1.19	0.847	0.2393	-
O_2 model using optimized fixed E_{O2}					
	Equation		**r^2**	**RMSE**	
E_{O2} 15 min					
Females	0.01254 * speed + 0.090		0.233	0.0135	
Males	0.00637 * speed + 0.146		0.028	0.0230	
COT		**AIC**			
Females	$0.210 * speed^2 - 1.007 * speed + 1.563$	-84.26	0.791	0.0937	2.2 – 2.7
	$0.7518* speed^{-0.8773}$	-89.45	0.805	0.0896	-
Males	$0.116 * speed^2 - 0.726 * speed + 1.392$	-321.24	0.847	0.0656	2.8 – 3.3
	$0.7813 * speed^{-0.9277}$	-323.29	0.847	0.0654	-
O_2 model including a set O_2 uptake curve					
	Equation		**r^2**	**RMSE**	
E_{O2} 15 min					
Females	$0.00601 * speed^3 + 0.0731$		0.904	0.0111	
Males	$0.00769 * speed^3 + 0.1050$		0.970	0.0140	
COT					
Females	$0.191 * speed^2 - 0.751 * speed + 1.161$		0.864	0.0323	1.7 – 2.2
Males	$0.109 * speed^2 - 0.470 * speed + 0.944$		0.713	0.0404	1.9 – 2.4

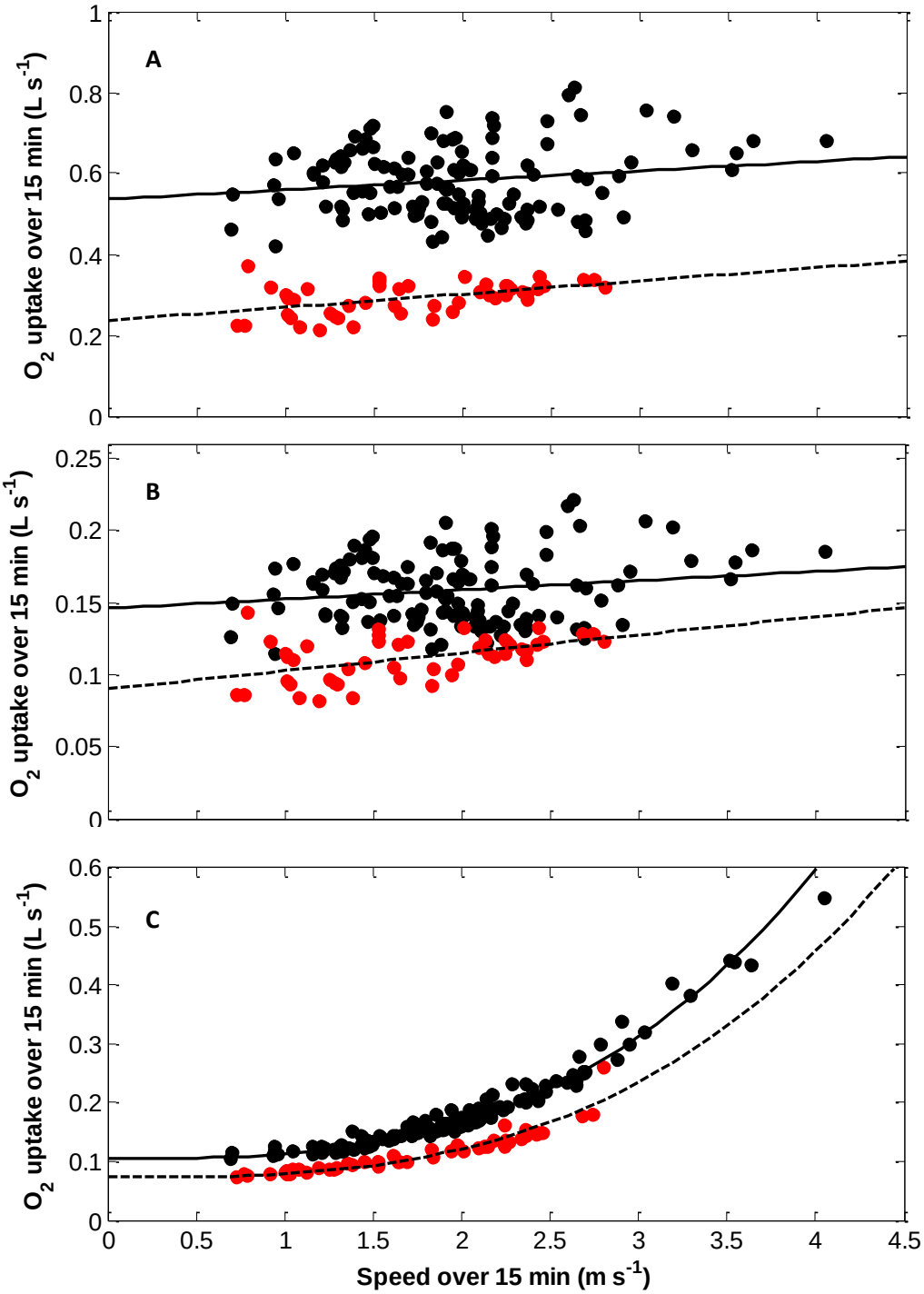

Figure 2.20. O_2 uptake over 15 min intervals as a function of speed over 15 min intervals estimated through the O_2 model including fixed O_2 uptake per breath (A), optimized fixed O_2 uptake per breath (B), and fluctuating O_2 uptake per breath according to the set O_2 uptake curve (C) for adult male (black) and female (red) Norwegian killer whales. Solid regression lines belong to males and dashed to females.

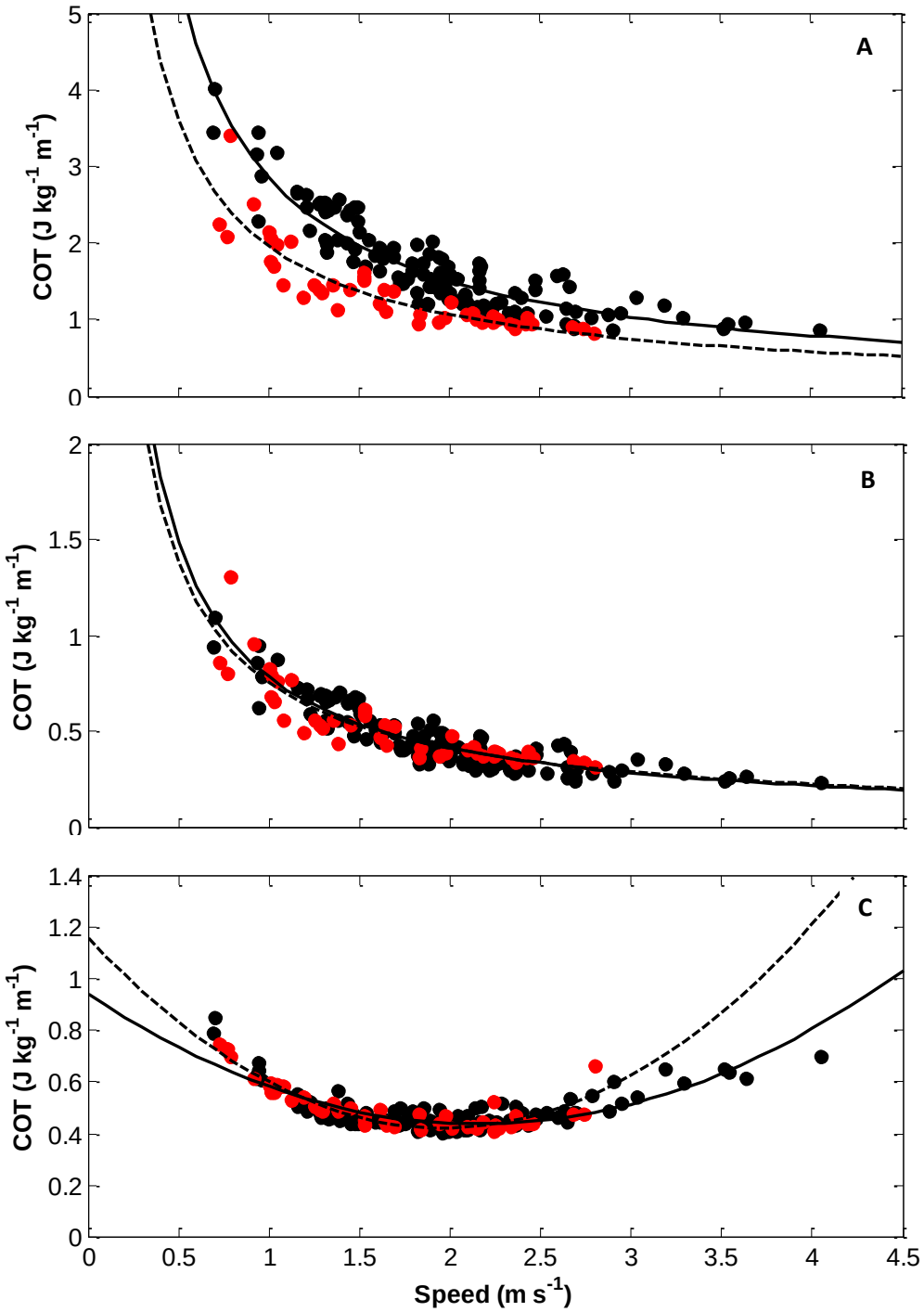

Figure 2.21. Mass-specific cost of transport (COT) as a function of speed estimated through the O_2 model including fixed O_2 uptake per breath (A), optimized fixed O_2 uptake per breath (B), and fluctuating O_2 uptake per breath according to the set O_2 uptake curve (C) for adult male (black) and female (red) Norwegian killer whales. Solid regression lines belong to males and dashed to females.

2.4. Discussion

The results of the present study showed that by applying an E_{O2} which fluctuates according to O_2 store at the time of each breath, a more realistic balance arose between estimated E_{O2} and V_{O2} over 15 min intervals in contrast to using a fixed E_{O2}.

2.4.1. Evaluation of the predictive power of the different O_2 models

The duration of tagging records differed per individual killer whale, so model fit was assessed according to the number of 15 min periods, which varied between individuals and categories. With the main goal of the present study to assess E_{O2} alternatives in relation to an O_2 model plus the fact that precise parameter values remain indeterminate for now, the focus of evaluation was on the overall contrasts between different O_2 uptake models rather than the specific metabolic rate estimates produced by the models.

Kriete (1995) and Williams and Noren (2009) measured horizontal speed using theodolites, whereas during the present study speed through water was derived from tag data and so care should be taken when comparing research outcomes. Still, since Kriete (1995) and Williams and Noren (2009) estimated E_{O2} with *in-situ* killer whales using respiration rates, outcomes of the present study were contrasted to their findings.

Williams and Noren (2009) fitted a linear function to respiration rate, which was multiplied with a fixed E_{O2} found by Kriete (1995) to estimate MR, versus speed, despite the conclusion drawn by Kriete (1995), in that respiration rates alone are not a reliable metric for energy demands. Christiansen *et al.* (2014) also fitted a linear function between respiration rate and speed. However, it has been suggested that MR increases non-linearly with speed due to drag (Kooyman, 1989), which was found for grey and minke whales using also respiration rate to estimate MR (Sumich, 1983; Blix and Folkow, 1995) and was also implemented in the overall O_2 model of the present study.

For the present study activity level and E_{O2} were averaged over 15 min so results could be compared to those of Kriete (1995) and Williams and Noren (2009). Implementation of shorter

time intervals would expose more of the variation in both speed and E_{O2}. However, due to the typical diving behaviour of killer whales, consisting of deep dives followed by breathing bouts, too small time intervals are not considered valuable for a realistic reflection of the correlation between activity and E_{O2}. Applying shorter time intervals would result in a weaker or no correlation between the two (Fig. 2.22).

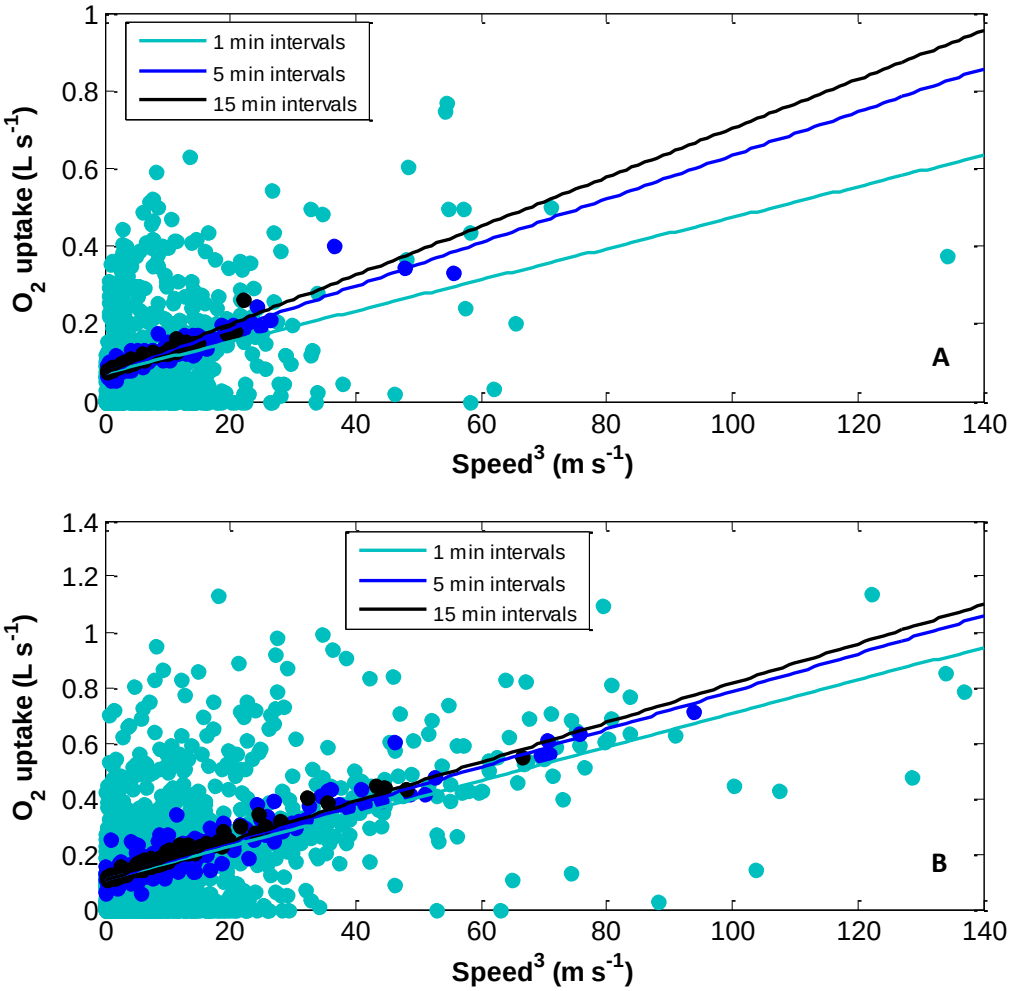

Figure 2.22. Oxygen uptake versus speed^3, both averaged over 1, 5 and 15 min intervals, estimated through the model with fluctuating O_2 uptake according to a set O_2 uptake curve for female (A) and male (B) killer whales. Solid lines represent the O_2 model fitted with intercept set at BMR for the implementation of 1 min intervals (females: r^2 = 0.100, RMSE = 0.1113, coefficient = 0.0040, males: r^2 = 0.280, RMSE = 0.1375, coefficient = 0.0060), 5 min intervals (females: r^2 = 0.873, RMSE = 0.0170, coefficient = 0.0056, males: r^2 = 0.875, RMSE = 0.0312, coefficient = 0.0068), and 15 min intervals (females: r^2 = 0.899, RMSE = 0.0112, coefficient = 0.0063, males: r^2 = 0.954, RMSE = 0.0161, coefficient = 0.0071).

2.4.1.1. The O_2 model including fixed and optimized fixed O_2 uptake per breath

Optimized fixed E_{O2} per category was considerably lower than the fixed E_{O2} value used by Kriete (1995) and Williams and Noren (2009). While the emphasis was laid upon modelling the E_{O2} versus the locomotion V_{O2} per 15 min intervals the model with the intercept set at BMR (including either the fixed E_{O2} as applied by Williams and Noren (2009), or the optimized fixed E_{O2}) did not correlate with speed^3 for (almost) any individual or category; estimated MR per 15 min was high and not in proportion to the BMR. The O_2 model with fixed optimized E_{O2} yielded a positive r^2-value for only one female and one male. Applying the GEEGLM per category with a set intercept was not accurate probably due to the impact of individual slope coefficients of correlations. While applying fixed E_{O2} without setting the intercept, model fit increased considerably for most females and females as category, yet BMR was 3.7 and 5.4 times higher than the BMR used for females and males, respectively, which is believed to be unrealistic (see Section 2.4.6.4 and 2.4.6.5). Moreover, only one male showed correlation between E_{O2} and speed^3.

When using the fixed E_{O2} value used by Kriete (1995) and Williams and Noren (2009), the estimated O_2 store kept increasing unrealistically high and unlimited, surpassing the estimated TBO capacity vastly. The E_{O2} was much greater than the V_{O2} per 15 min interval according to speed^3 and the slope of MR_L vs. speed^3 used (Appendix 2.2). O_2 store estimated using the optimized fixed E_{O2} obviously resulted in a balance between E_{O2} and V_{O2} over the entire tag records, yet within records estimated O_2 store occasionally surpassed the TBO capacity or became negative (Appendix 2.2). Using an optimized fixed E_{O2} the model did produce considerable lower uptake estimates over 15 min intervals plus reduced the difference between E_{O2} estimates for males and females compared to the fixed uptake as used by Williams and Noren (2009). The optimized fixed E_{O2} values estimated for males and females according to the O_2 model were 3.7 and 2.6 lower, respectively, than the fixed E_{O2} as used by Kriete (1995) and Williams and Noren (2009). Kriete (1995) handled the maximum V_T found rather than the average one since she expected this value was more comparable to the V_T of free-ranging killer whales. Yet, in relation to mean V_T measured fixed E_{O2} would be 17.9 and 6.2 L for males and females, respectively (Kriete, 1995). These values are still 194 % and 36 % higher than the optimized fixed E_{O2}, for males and females, respectively. So apart from the fact that according to the model the fixed E_{O2} is expected to overestimate energetic requirements

of both sexes, the overestimation of males is higher than for females.

Williams and Noren (2009) claimed to have found a linear correlation between respiration rate, and therefore MR, and speed, with rather low r^2 values of 0.10 and 0.26 for males and females, respectively. Christiansen *et al.* (2014) found an even weaker linear correlation between breathing rate and swimming speed for minke whales (r^2 = 0.065).

Thus, since there was no balance between E_{O2} and V_{O2} over 15 min intervals for applying either a non-optimized or optimized fixed E_{O2} per breath to the O_2 model in combination with the tag data, this study showed that breathing rate alone does not appear to enable accurate estimations of energy consumption.

2.4.1.2. The O_2 model including a set O_2 uptake curve

E_{O2} estimated by the O_2 model with fluctuating E_{O2} correlated with speed^3 significantly better than applying the optimized fixed E_{O2} for all individuals and categories. Having the E_{O2} fluctuate according to the O_2 store at time of each breath, depending upon apnea duration and MR during apnea, led to a much stronger correlation between estimated E_{O2} and level of activity over 15 min. Thus, this model variant yielded a better balance between V_{O2} and E_{O2} over the entire tag record. The maximum possible uptake affected the O_2 uptake of the first couple of breaths after long apnea most strongly, due to low O_2 store at those times. E_{O2} during subsequent breaths was simply whatever was required to replenish the O_2 storage fully.

It was hypothesized that the last breaths in a breathing bout do not have the purpose to take up O_2, but rather offload CO_2 (Wilson *et al.*, 2003). O_2, the primary limiting factor in optimal diving models, is a necessity for body activity. Yet, it has been revealed that accumulation of CO_2 during apnea is potentially just as important as a respiration cue (Gallivan, 1980; Butler, 1982; Halsey *et al.*, 2003; Green *et al.*, 2005; Fahlman *et al.*, 2008a; Houston, 2011; Noren *et al.*, 2012b). Current results indicated decreasing re-oxygenation efficiency per breath throughout breathing bouts following longer dives. Whales kept breathing when O_2 stores were predicted to be replenished, possibly to offload CO_2 (Boutilier *et al.*, 2001).

It appeared that the average E_{O2} per breath estimated over the 15 min intervals by the O_2

model with a set O_2 uptake curve was quite a bit lower than the fixed E_{O2} value used by Kriete (1995) and Williams and Noren (2009), espcially for males. Estimated MR values were overall more similar to the ones projected by the O_2 model with the optimized fixed E_{O2} than with the non-optimized fixed E_{O2}, as expectted. Yet, there was a clear linearly increasing correlation between estimated E_{O2} and speed^3 applying a set O_2 uptake curve to the model, whereas for the optimized fixed E_{O2} model there was almost no increase of estimated E_{O2} with speed^3.

When applying a set O_2 uptake curve to the O_2 model, female 05_322a showed a distinct difference in correlation between estimated E_{O2} and speed^3 over 15 min intervals, identifiable as the observation with highest speed. This female had the steepest slope for estimated E_{O2} versus activity, meaning she used more O_2 compared to the other females for any given speed. Meantime, she exhibited the strongest correlation between estimated E_{O2} and speed^3 over 15 min intervals, and even without her extreme observation the slope of the correlation would still be higher than for all other females. However, she was seen travelling with a calf. The fact that when using the optimized fixed E_{O2} in the model this individual would not have been indicated as an extreme case showed how different the model outcomes were depending on whether timing of breath was taken into account or not.

It could be anticipated that values for the parameters applied to the O_2 model with a set O_2 uptake curve were reasonably in relation to one another as the estimated O_2 store did not decrease to be unrealistically low, and a balance between E_{O2} and V_{O2} existed. If E_{O2} and V_{O2} values did not relate to one another, a great decrease of store would indicated that either the maximum E_{O2} was too low to replenish the O_2 depletion or V_{O2} use would be too high due to the slope of MR_L vs. speed^3. Also O_2 store could be too high or too low, which was not apparent.

In summary, including a set O_2 uptake curve within the O_2 model proved to be an essential improvement in comparison to the O_2 model with a fixed E_{O2} for estimating energy demands in adult killer whales. However, it should be stressed that this model is a first prototype and effort should be put into producing a more complete model of gas exchange across different animal compartments.

2.4.1.3. The O_2 model including an optimized O_2 uptake curve

The O_2 model including an optimized O_2 uptake curve fitted better to speed^3 than the model including the non-optimized uptake curve for four of the females and three of the males. However, results showed that optimization was sensitive to random effects by individuals. And though these effects were taken into account during modelling, the optimized curve for females as category deviated from optimized curves found for individual females. Then again, this study represents only a simplified O_2 model on which many aspects could be improved and extended. Consequently, the optimization of the O_2 uptake curve was just a first impression of how the relationship between E_{O2} per breath and TBO potentially varies per individual whale. Differences in optimized curve shape were likely triggered by a combination of V_{O2} and apnea duration, and possibly by biological and ecological factors.

For individuals and categories showing a horizontal asymptote for r^2 versus fitted exponent values, the slope of this r^2-exponent relationship differed. This slope affected the exponent value for which the threshold of 0.5 % of the r^2 variance was reached. Still, it was expected that for all individuals and categories for which an asymptote was observed, the O_2 model including the non-optimized O_2 uptake curve fitted best. It could be argued that the presence of an asymptote indicated that a whale required a higher maximum E_{O2}. However, this was unachievable to determine as an increase in maximum E_{O2} would increase model fit by itself as discussed before. Both biological and ecological factors could affect O_2 use per individual as discussed later.

It was reasonable that for both models including fluctuating E_{O2} the correlations of V_{O2} and speed^3 were similar; both maximum E_{O2} and the slope of MR_L vs. speed^3 were set to be the same. Thus the estimated E_{O2} over the same 15 min intervals were comparable, only the E_{O2} uptake per consecutive breath differed somewhat. For the individuals without an asymptote, the O_2 model with the optimized curve led to a stronger correlation between V_{O2} and speed^3 over 15 min periods since E_{O2} per breath decreased gradually and smoothly with increasing O_2 store, whereas the non-optimized curve showed a sharp bend. This caused the E_{O2} to vary more accurately in relation to the O_2 store and produced consequently a somewhat tighter correlation between E_{O2} and speed^3, or V_{O2} during 15 min intervals. For any O_2 store, except 0, E_{O2} according to the optimized uptake curve was lower than E_{O2} according to the non-

optimized set curve.

Applying an optimized O_2 uptake curve to the O_2 model showed once more that the relationship between E_{O2} and O_2 store is most certainly not a horizontal line and that therefore respiration timing is important in estimating energetic requirements of killer whales.

2.4.2. Speed from flow noise as an activity metric

The overall results revealed that speed derived from flow noise recorded with DTAGs had potential to be used as activity metric in the proposed O_2 model. Speed was measured as the depth change per time, and therefore when a whale was swimming vertically this speed measurement was more accurate than when a whale was swimming at a shallow pitch angle. Still, when plotting speed versus SPL, there was variation of the measurements around the final relationship, which was merely a best approximation of the true relationship between speed and SPL.

Speed was uniquely estimated per individual in consideration of tag placement and tag movement during deployment was considered to account for potential changes in correlations between SPL and speed within one individual. However, influence on speed estimations by constant ocean ambient noise, noise generated by oscillation of the flukes (depending on tag placement) and noise generated by the water surface recorded for logging killer whales, which were all considered insignificant to the present study aims, are worthy to be examined in future research.

2.4.3. Respiration events

In some rare events there could have existed false absences or presences concerning the extraction of breathing events from tagging records due to both manual and automatic errors, especially during logging periods exhibited occasionally by female 05_322a. Though these false exclusions or inclusions of breaths were expected to affect the O_2 uptake for a small number of succeeding breaths, this should not significantly influence the model outcomes based on the 15 min intervals.

2.4.4. Ecological and biological influences on respiration behaviour

Results of the O_2 model including fluctuating E_{O2} showed that there existed not only differences in the correlation between E_{O2} and speed^3 between sexes, but also between individuals within a sex category. Diving behaviour is shaped by the individual itself, influenced not only by physiological, but also biological, behavioural, and ecological factors.

Individuals which were classified as adult female, according to the attendance of a calf or juvenile, could have included sub-adult, pre-sprouting males as no DNA was collected for gender confirmation. This uncertainty should be considered when reflecting other aspects, such as the influence of calf presence. Kriete (1995) and Williams and Noren (2009) found a statistically higher respiration rate for females for all speeds observed. The latter authors explained this finding by the fact that females have a higher mass-specific MR due to smaller body size. However, the present study showed that it was inaccurate to draw such conclusions from estimating MR from solely breathing rates. Moreover, though mass-specific MR estimated through the O_2 model with a set O_2 uptake curve was higher for females than for males, there existed no statistical evidence that sex in killer whales, in addition to speed, had an influence on respiration rate.

Baird *et al.* (2005) found that adult male killer whales dove deeper more frequently than females and concluded that this difference in diving behaviour in fish-eating killer whales is triggered to minimize food competition with pod members reflecting diving capacities difference regarding body size. In transients, Miller *et al*. (2010) found no difference in breathing rates between juveniles, adult females, and adult males.

Female energetic requirements are higher during lactation (Fedak and Anderson, 1982; Costa *et al.*, 1986; Kriete, 1995; Noren *et al.*, 2011) and by increase in drag due to echelon position swimming by a calf (Noren, 2008). The higher E_{O2} of female 05_322a could potentially be explained as she was seen travelling with a calf. Female 06_313s and 06_327s were travelling with a juvenile and small animal, respectively. However, as these two females exhibited the two lowest slopes between E_{O2} and speed, it could be suggested that older infants were causing negligible MR elevation as they were more independent than female 05_322a's calf.

Implementing ecological factors in optimal diving models per individual is a challenge. Habitat features can impact foraging behaviour; herring-eating killer whales tend to adapt their foraging strategy depending on water depth (Nøttestad and Similä, 2001). The variation in prey distribution and abundance influences the diving behaviour of the predator either on a long- (seasonal) or small-time (daily) scale (Kramer, 1988; Baird *et al.*, 2005; Baird *et al.*, 2006; Miller *et al.*, 2010). Perceptibly, it is not only the prey occurrence but also the foraging or hunting strategies that will be decisive for respiration timing (Thompson *et al.*, 2003). Species which are social and behave in groups, such as killer whales, are expected to be partly constrained by conspecifics' decision-making. Within the 12 studied killer whales there existed four couples which were tagged simultaneously, of which three couples belonged to the same social group (Table 2.3). Movements, including respirations, within matrilines show greater synchronicity compared to with other subgroups in a pod (Ray *et al.*, 1986). Variation in age and sex will create variation in physiological abilities between group members and so some will have to push their limits more than others, especially during common hunting (Noren, 2008; Costa, 2009; Miller *et al.*, 2010). In all social groups to which the tagged individuals belonged at least one juvenile or calf was present. However, to account for this difference in diving capacities Miller *et al.* (2010) suggested that either larger individuals could perform under their physiological maximum to meet the smaller whales with physiological constraints (physiological compromise), or hunting and foraging roles could be assigned to individuals according to physiological capacities (division of labour). Social behaviour is challenging to integrate in a model for diving behaviour of an individual. Consequently, diving experiments done on solitary animals *in vivo* should be interpreted with caution in case this is in fact a social animal while at sea.

2.4.5. Metabolic cost of transport

Unrealistically, COT estimated through the O_2 model with both fixed E_{O2}'s kept decreasing with increasing speed. Williams and Noren (2009) and Christiansen *et al*. (2014), both found a linear correlation between breathing rate and speed, and argued that the absence of a minimum COT was caused by the lack of high speed observations. However, when COT was estimated by the O_2 model with a set O_2 uptake curve, the expected curvilinear relationship between MR and speed was found, showing a clear minimum between approximately 1.7 and 2.2 $m \cdot s^{-1}$ for females and 1.9 and 2.4 $m \cdot s^{-1}$ for males. For other studies inferring a curvilinear correlation

between E_{O2} or breathing rate a clear minimum COT was found as well (Sumich, 1983; Williams *et al.*, 1993; Kriete, 1995; Yazdi *et al.*, 1999; Otani *et al.*, 2001). Consequently, it can be concluded that it was not the lack of high speed observations, but the shape of regression fitted between E_{O2} and speed which determined either the absence or presence of a minimum COT.

The mean speed of 1.7 and 2.0 $m \cdot s^{-1}$ found during the present study for females and males, respectively, fell within the optimal COT speed ranges (Fig. 2.21). Average speed observed by Williams and Noren (2009) of 1.7 and 1.5 $m \cdot s^{-1}$ for males and females, respectively, was lower than optimal speed ranging from 2.6 to 3.0 $m \cdot s^{-1}$ for both sexes estimated in that study, possibly due to overestimation of optimal speed as explained above. However, the speeds observed by Williams and Noren (2009) were very similar to optimal speed estimated during the present study. The optimal COT speed derived here was lower than the optimal speed of 3.1 $m \cdot s^{-1}$ for female and male killer whales found by Kriete (1995). Nonetheless, she observed a mean speed of approximately 2.0 $m \cdot s^{-1}$ for both sexes which were very similar to the observed and estimated optimal speed of the present study. These latter speeds were also similar to results on other cetacean species (Sumich, 1983; Williams *et al.*, 1992; Williams *et al.*, 1993).

Estimated minimum mass-specific COT according to fluctuating E_{O2} was 0.44 and 0.42 $J \cdot kg^{-1} \cdot m^{-1}$ for male and female killer whales, respectively. According to the correlation between body mass and minimum COT found by Williams (1999), based on pinnipeds and bottlenose dolphins, a male and female killer whale would have a higher minimum COT of approximately 0.71 and 0.78 $J \cdot kg^{-1} \cdot m^{-1}$, respectively. Kriete (1995) estimated similar minimal COT of 0.75 and 0.83 $J \cdot kg^{-1} \cdot m^{-1}$ for males and females, respectively (Fig. 2.23). However, Williams and Noren (2009) found considerably higher minimum COT of approximately 1.1 and 0.95 $J \cdot kg^{-1} \cdot m^{-1}$ for males and females, respectively (Fig. 2.23). Still, for the two latter studies MR was estimated through the non-optimized fixed E_{O2} found by Kriete (1995), which was expected to overestimate true MR and so also minimum COT. Minimum COT values found during the present study lay under the linear model fitted to available data on minimum COT including values of various other cetacean species (Fig. 2.23). Yet, when excluding the minimum COT estimated by Kriete (1995) and Williams and Noren (2009), assuming that these would indeed overestimate minimum COT, the estimates of minimum COT of the present study would lay considerably closer to the fitted linear model (Fig. 2.23). Reasons for the found diverged

minimum COT values in killer whales remain unknown. Moreover, for concrete values on MR were undetermined no firm conclusions could be drawn according to found minimum COT values.

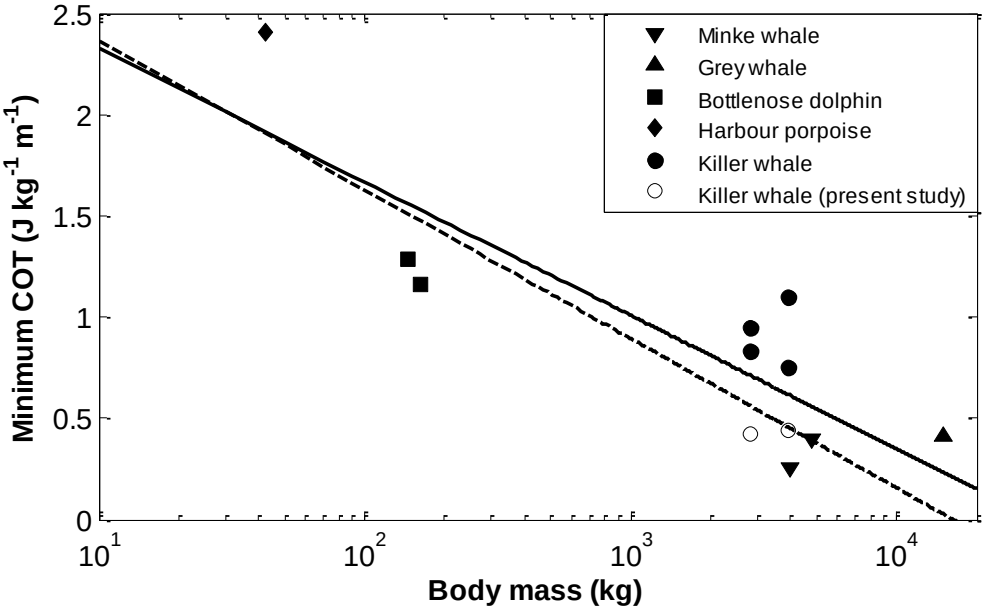

Figure 2.23. Estimated minimum mass-specific cost of transport (COT) in relation to body mass for various cetaceans during previous studies. Minimum COT is shown for the harbour porpoise (Otani *et al.*, 2001), bottlenose dolphin (Williams *et al.*, 1993; Yazdi *et al.*, 1999), killer whale (Kriete, 1995; Williams and Noren, 2009; present study), minke whale (Blix and Folkow, 1995; Christiansen *et al.*, 2014), and grey whale (Sumich, 1983). The solid line represents the simple linear regression fitted to all data: minimum COT = 2.98 – 0.286 * log(body mass) (r^2 = 0.722, RMSE = 0.3294). The dotted line represents the simple linear regression fitted to the same data, yet excluding the minimum COT estimated for killer whales by Kriete (1995) and Williams and Noren (2009): minimum COT = 3.10 – 0.319 * log(body mass) (r^2 = 0.839, RMSE = 0.3207) (graph adjusted from Christiansen *et al.* (2014)).

2.4.6. Physiological aspects

The results of this thesis were partially based upon the physiological parameters applied to the O_2 model of which exact values regarding killer whales were still uncertain at the time of this study. Sensitivity analyses were carried out for the most uncertain parameters, which showed that the O_2 model is most sensitive for variation in the slope of MR_L vs. speed^3. Female data was more sensitive to value change than the male data.

2.4.6.1. Body size

Sexual dimorphism in killer whales, with males being larger than females, drives diving physiology and capabilities between genders (Clark and Odell, 1999; Baird *et al.*, 2005; Ford, 2009; Miller *et al.*, 2010). In this study all physiological parameter values were attuned to one typical body mass for an adult male and a female killer whale measured indirectly from captive north eastern pacific resident herring-feeding specimens by Kriete (1995). Potential body size differences between the individual studied by Kriete (1995) and the studied Norwegian killer whales could not have affected the main study results. Though especially adult males included in the present study showed difference in body mass, this feature was unfeasible to measure from the *in-situ* tagged individuals. It would be valuable examining precise influence by body mass on the O_2 model through replicating the present study on captive specimens and/or measurement of body sizes of tagged animals in future studies.

Males 05_320b and 05_322b exhibited the highest slopes for the correlation between estimated E_{O2} and estimated V_{O2} for over the 15 min intervals, meaning they had taken up more O_2 than other males for any given speed. A potential explanation for this fact could be that they had experienced more drag due to their larger body size and therefore had a higher MR than was estimated in this study.

2.4.6.2. O_2 store capacity

Greater body size volume generates a greater O_2 store capacity (Kooyman *et al.*, 1983; Noren and Williams, 2000; Noren *et al.*, 2012b). During the present study the mass-specific TBO was assumed to be 35.7 ml $O_2 \cdot kg^{-1}$, as measured by (Noren *et al.*, 2012b). This TBO value was comparable to that found for the bottlenose dolphin (Williams *et al.*, 1993) and the California sea lion (Feldkamp *et al.*, 1989), but lower than for the beluga whale (Shaffer *et al.*, 1997), harbour seal (Davis *et al.*, 1991), and Weddell seal (Ponganis *et al.*, 1993). If true TBO would be higher than as set in the O_2 model, yet MR and maximum E_{O2} stayed the same, the chance to reach a negative O_2 store would decrease. With a negative O_2 store the whale would take up maximally during breathing, as for when the O_2 store would be less than the TBO minus the maximum E_{O2}. Therefore, it was expected that change in O_2 store capacity would not greatly affect the main study results.

2.4.6.3. Maximum O_2 uptake per breath

Knowledge regarding thorough numbers on E_{O2} during respiration in cetaceans, especially larger species including the killer whale, was absent at the time of the present study. Despite significant uncertainties, precise fixed E_{O2} per breath were assigned during all preceding studies on estimating energetics for free-ranging cetaceans through respirations, showing a remarkably large range between different baleen species from 11 % for grey - and humpback whales (Sumich, 1983; Dolphin, 1987) to 45 % for minke whales (Blix and Folkow, 1995; Christiansen *et al.*, 2014). However, Blix and Folkow (1995) combined values from studies on a small cetacean (bottlenose dolphin) and values from studies on a large baleen whale calf (grey whale), from which they also used data on V_T. These species are non-comparable when it comes to body sizes and behavioural patterns and therefore pooling data from the two can potentially create incorrect E_{O2}values. Kriete (199) and Williams and Noren (2009) employed a fixed E_{O2} of 41 and 38 % for adult male and female killer whales, respectively. However, according to the presented O_2 model applied with fixed E_{O2}, optimized percentages were 11 and 15 % for males and females, respectively.

Nevertheless, the key feature of the alternative O_2 model was the fluctuating E_{O2} for which the maximum was set according to measurements made for killer whales by Kriete (1995) at 47 and 44 %, for males and females, respectively, assuming steady V_T of 258.5 and 149.0 L, respectively (Kriete, 1995). Though during all preceding *in-situ* studies on cetacean energetics E_{O2} was assumed to be constant, large variations in E_{O2} during respiratory cycles were observed for captive specimens ranging from as low as 8 % to more than 80 % (Irving *et al.*, 1941; Olsen *et al.*, 1969; Ridgway *et al.*, 1969; Sumich, 1994; Kriete, 1995), depending on activity level, breath interval and breath number in sequence. Yet, not many studies focussed on determining a maximum E_{O2} efficiency according to apnea, (Ridgway *et al.*, 1969) found a maximum E_{O2} of 86 % for a first breath in a bottlenose dolphin after long apnea at the surface. Kasting *et al.* (1989) found a maximum E_{O2} very similar to the one used in the current O_2 model of 44 % for the same adult male killer whale Kriete (1995) studied. According to the E_{O2} efficiency of 86 % found by Ridgway *et al.* (1969), an adult male and female killer whale would be able to take up 46.57 L and 26.85 L, respectively, during one breath, which is within the upper limits of the performed sensitivity analyses. The lowest value for these sensitivity analyses was set at less than half this percentage, with 35 % and 37 % for males and females, respectively.

During this study it was assumed that E_{O2} during breathing was related to the TBO deficit. However, apart from the variation in proportion of the inhaled O_2 extracted by the lungs, the proportion of O_2 diffused from the lungs into the blood during apnea can vary simultaneously (Olsen *et al.*, 1969; Kriete, 1995), with excess O_2 being exhaled before inhalation of fresh air. To account for this concern mean E_{O2} instead of maximum E_{O2} were taken from Kriete (1995) to multiply with max V_T to estimate the maximum possible E_{O2}. Besides, E_{O2} information collected from captive specimens should be handled with care concerning the stress experienced by the animals and its influence on measurements. Given these general uncertainties concerning E_{O2}, the sensitivity analyses results are important. These showed that the correlation between E_{O2} and V_{O2} over the 15 min intervals got tighter with increase in maximum E_{O2}. This can be explained by the fact that when the maximum possible uptake would be equal to the TBO capacity, a whale would replenish its O_2 store completely with every breath. Still, a value this high is physiologically unrealistic regarding V_T: the maximum V_T of 258 L measured by Kriete (1995) for an adult male killer whale contained 54 L of O_2. However, for females there was a considerable increase in model fit by increasing the maximum E_{O2} compared to males and therefore it could be argued that the applied value for this category was somewhat low. In contrast, a whale would not be able to replenish its O_2 storage when maximum E_{O2} is too low. According to the sensitivity analyses outcomes there was no indication of a most realistic approximation of the maximum E_{O2} in killer whales, however it can be concluded that for the large range of maximum E_{O2} values applied, the O_2 model including fluctuating E_{O2} constantly produced a more realistic balance between the E_{O2} and V_{O2} over the 15 min intervals.

2.4.6.4. Basal metabolic rates

Basal metabolic rates are a challenge to measure even in captivity, not only due to the size of large cetaceans but also due to the set criteria by Kleiber (1975) especially regarding resting. Consequently, few data on BMR in killer whales existed at the time of the study. For it is assumed that a cetacean continuously exhibits a somewhat active state to come to the surface to breath even though it is resting (Brodie, 1975), it was chosen to refer to standard metabolic rate with BMR.

The few data available on killer whale BMR indicated large variation. According to the equation

founded for killer whales by Kasting *et al.* (1989) BMR would be 2.7 times higher than the values used for both males and females. Also during other studies on killer whale BMR it was concluded that this value was higher than predicted by the Kleiber (1975) regression (T.M. Williams, personal communication). Guinet *et al.* (2007) used the equation provided by Motani (2002) for killer whale BMR estimation, which took into account an elevated BMR according to the body mass in cetaceans compared to the equation for terrestrial animals provided by Kleiber (1975). According to this equation BMR was 5 % higher (0.0999 $LO_2 \cdot s^{-1}$) and 8 % lower (0.0792 $LO_2 \cdot s^{-1}$) than the values used for males and females, respectively. Extrapolated BMR measured for an adult male killer whale by Worthy *et al.* (2013) yielded a BMR of respectively 21 % (0.0833 $LO_2 \cdot s^{-1}$) and 19 % (0.0595 $LO_2 \cdot s^{-1}$) lower than the values used for males and females, respectively. According to the correlations found between respiration rate and speed for free-ranging killer whales by Williams and Noren (2009) BMR would be approximately 4.6 (0.4832 $LO_2 \cdot s^{-1}$) and 3.0 (0.21817 $LO_2 \cdot s^{-1}$) times higher than the values used for males and females, respectively.

Even though exact values for BMR remain undefined, the sensitivity analyses indicated that this uncertainty did not cause change of the main study results in that applying fluctuating E_{O2} to the model according to O_2 store at time of the breath leads to a stronger relationship between V_{O2} and activity levels.

2.4.6.5. Metabolic rates

As for the other model parameters, there was no concrete data available for MR as a function of speed for killer whales and therefore uncertainty regarding this value existed. Of all parameters, the O_2 model appeared most sensitive to variation in the slope of MR_L vs. speed^3, with slope of the correlation over 15 min periods notably changing according to the variation of this value for the lower tested values. In relation to the sensitivity analyses outcomes it could be argued that for females the slope of MR_L vs. speed^3 was set too high; the relationship between E_{O2} and V_{O2} over the 15 min intervals was not as strong as for a lower slope of MR_L vs. speed^3. However, according to measurements made by Kriete (1995) a captive adult male and female killer whale had a MR of 0.595 and 0.492 $LO_2 \cdot s^{-1}$, respectively, during a show, implying mean V_{O2} and breathing rate and maximum V_T. To produce a same MR using Eq. 10, a male and female would have had a speed of 4.3 and 4.4 $m \cdot s^{-1}$, respectively, which is a realistic average

speed for during a show; Guinet *et al.* (2007) measured a maximal speed in free-ranging killer whales of 5.6 $m \cdot s^{-1}$ while chasing tuna.

According to the equations used in the present study to establish the V_{O2}-speed^3 relationship, C_D ranged between 0.0138 and 0.0228 for females, and 0.0111 and 0.0215 for males, according to their minimum and maximum speed measured over 15 min intervals. Fish (1998) found a theoretical C_D of 0.0026 for a Re of $3.65 \cdot 10^7$ for killer whales, which is 3.8 times as low as by calculating C_D using Eq. 7. If the C_D found by Fish (1998) was true, then using Eq.7 would have yielded a V_{O2} of 3.8 too large, and the overestimation of energetic requirements estimated through respiration rate would be even more drastic. However, Guinet *et al*. (2007), using a similar theoretical approach as Fish (1998) to define MR in killer whales, found a V_{O2} of 0.02 $LO_2 \cdot kg^{-1} \cdot min^{-1}$ for a swim speed of 4.7 $m \cdot s^{-1}$. This would mean that a female killer whale (2,800 kg) of the present study had a V_{O2} of 0.8886 $LO_2 \cdot s^{-1}$ for the same swim speed. Whereas, according to the MR_L vs. speed^3 slope used in the present study a female swimming at a similar speed would have a V_{O2} 1.6 times as low (0.5481 $LO_2 \cdot s^{-1}$). This aspect indicates once more the uncertainty in predicting actual MR in killer whales.

The relationship between Re and the C_D, as found for killer whales by Fish (1998) and used in the present study to estimate the slope of MR_L vs. speed^3, was based on the unsteady wing theory, accounting for drag by actively swimming movements. Doing so, the C_D varied according to swimming speed, which is related to body swimming movements (Fish, 1998). During other previous studies on drag in swimming animals it was shown that the so-called active drag could be 3-7 times higher than the passive drag (Lighthill, 1971; Fish *et al.*, 1988). Understandably, firm values of drag forces caused by propulsions remain difficult to obtain. Sensitivity analysis of the present study indicated that significant increase of the slope of MR_L vs. speed^3, would not improve the correlation between V_{O2} and speed^3 over 15 min intervals.

Data available on MR measured for other cetaceans showed great variation, with all of them being relatively considerably higher than the estimates upon which the O_2 model is based and which were derived using the same method as used by Guinet *et al.* (2007). Highest estimated speed during the present study was 4.1 $m \cdot s^{-1}$ and 2.8 $m \cdot s^{-1}$ for males and females, respectively, with corresponding MR of 0.527 and 0.181 $LO_2 \cdot s^{-1}$, respectively. When increasing the slope of MR_L vs. speed^3 in the O_2 model with a set O_2 uptake curve to one of the two highest values

tested during sensitivity analyses (found by Williams *et al.* (1993) for bottlenose dolphins) O_2 store would become so low that a whale would take up O_2 maximally during every single breath without being able to replenish its store, which is unrealistic. Despite the fact that smaller cetaceans are expected to have a higher mass-specific MR compared to larger cetaceans (Costa and Williams, 1999; Boyd, 2002; Costa, 2009), the corresponding extrapolated MR for males and females killer whales of 5.58 and 1.29 $LO_2 \cdot s^{-1}$, respectively, for maximal speed is expected to overestimate true MR. In relation to MR measurements made by Otani *et al.* (2001) on harbour porpoises a male and female killer whale would exhibit a MR of around 15.867 and 3.961 $LO_2 \cdot s^{-1}$, respectively, at the highest measured speed, which is as well high compared to the corresponding MR found during this study. According to measurements for beluga whales by Shaffer *et al.* (1997) an adult male killer whale would have a MR of around 1.471 $LO_2 \cdot s^{-1}$ during maximal speed. The beluga whale is on average about half the size of a killer whale (O'corry-Crowe, 2009) and thus has a higher mass-specific MR which could explain a higher slope of MR_L vs. speed^3 per kg. Though beluga whales are known for being poorly adapted for swimming at high speed (Shaffer *et al.*, 1997) this still does not explain the elevated MR compared to the ones found during this study for killer whales. On the contrary, MR found for average speed of the killer whales in the present study was 0.1507 $LO_2 \cdot s^{-1}$ and 0.0980 $LO_2 \cdot s^{-1}$ for males and females, respectively, and appeared in proportion with the average MR for slightly larger body sized minke whales (Perrin and Brownell, 2009) found by Folkow and Blix (1992), which was 0.1817 $LO_2 \cdot s^{-1}$.

Thus, though most MR data collected on other cetacean species were higher than the MR estimated through the slope of MR_L vs. speed^3 as used in the O_2 model of the present study, these did not improve the correlation between V_{O2} and speed^3 over 15 min intervals when applied to the O_2 model in combination with the other used parameters.

2.4.7. Conclusions and future work recommendations

The assumption that respiration rate is an appropriate proxy for the level of metabolic activity lays the foundation of an important part of fundamental research on the energetics of cetaceans, yet results presented here indicate its accuracy is open to question. The findings of the present study support the essential conclusion drawn previously by Sumich (1994) and Kriete (1995): respiration rate alone, ignoring breath-by-breath variation in O_2 exchange, is not

a reliable metric for producing energetic demand estimates in marine mammals. This study confirmed that respiration timing is crucial for deriving energetic estimations for killer whales from breathing observations; respiration timing in combination with the MR during a breath interval demarcated the O_2 store at the time of each breath, which in turn defined the O_2 uptake per executive breaths.

The fixed E_{O2} found by Kriete (1995) applied to estimate MR from breathing rates by Williams and Noren (2009) resulted in a substantial overestimation of energetic requirements for killer whales compared to the estimates derived by the O_2 model with an O_2 uptake curve. This observation questions the utility of using fixed E_{O2} for studying cetacean energetics in general, and estimations derived using this method should be questioned and handled with care when drawing firm conclusions. Inaccurate energetic estimations potentially have great influence on estimated food requirements by cetaceans and the evaluation of their impact on marine ecosystems as top predators.

Still, it should be stressed that though the presented O_2 model including a set O_2 uptake curve is an improvement on deriving energetic estimates compared to using a fixed E_{O2}, the model is not yet complete and various aspects of this model should be improved. First, the O_2 model should be validated through quantifying kinematics, O_2 usage and breath-by-breath E_{O2} and V_T from captive killer whales tagged with DTAGs. Blow-expirate of individual sequential breaths of tagged whales should be captured (Kriete, 1995) to quantify gas-exchange (V_{O2} and maximum E_{O2}) and V_T's per breath throughout breathing bouts, while locomotion effort of captive whales is quantified through stroking rate and speed (Goldbogen *et al.*, 2006; Gleiss *et al.*, 2011; Simon *et al.*, 2012). The relationship between E_{O2} and O_2 store at the time of each breath is the key feature of the model; therefore, it is important to take into account aspects that potentially have impact on this curve shape.

During the present and most former studies on *in-situ* cetacean energetics V_T was assumed to be constant across breaths. Yet, V_T within an individual is not necessarily constant and has been shown to fluctuate (Spencer *et al.*, 1967; Olsen *et al.*, 1969; Wahrenbrock *et al.*, 1974; Gallivan *et al.*, 1986; Kriete, 1995). Results of this study showed that after long apnea the first breath is most important for O_2 store replenishment. Besides, it has been speculated that divers, including marine mammals, prepare for the succeeding dive by taking up extra O_2 if

required (Wilson, 2003; Wilson *et al.*, 2003; Wilson and Quintana, 2004; Baird *et al.*, 2006).

The rate at which O_2 is taken up into the body is not constant and relies not only on the O_2 partial pressure in the lungs, but also the O_2 partial pressure in the blood and muscles (Butler and Jones, 1997). In deep-diving cetaceans blood is the major O_2 store, while in other cetaceans the three stores are more closely equal (Kooyman, 2002; Ponganis, 2011). V_{O2} during prolonged periods of apnea in non-deep-diving cetaceans decreased non-linearly, indicating that the key O_2 supply will shift from the lungs to the blood and muscle tissues (Kramer, 1988; Gallivan, 1992; Kriete, 1995; Noren *et al.*, 2012b). Moreover, after the respiratory tract is reloaded by breathing, the blood and muscle stores will be refilled (Kriete, 1995). This reloading of the compartment during different time intervals is expected to have a significant influence on the true O_2 uptake curve. Simultaneously, due to lung collapse at depth, arterial partial pressure of O_2 increases swiftly early in a dive, followed by a fast decline as gas exchange from the lungs is hindered (Fahlman *et al.*, 2009). Also, marine mammals potentially have the option to voluntarily switch between O_2 stores using selective ischemia to muscles (Scholander, 1940). It is acknowledged that the established O_2 model should be extended into a more complete gas-exchange model by comprising the different body tissues and lung collapse (Fahlman *et al.*, 2006). Still, it is not foreseen this aspect would significantly influence the main outcomes of this study.

For this same reason anaerobic metabolism was not considered for this O_2 model; estimated O_2 store was allowed to become negative, but lactate built up was not explicitly taken into account. Blood lactate level increases due to the depletion of muscle O_2 stores triggered either by exceeding the aerobic breath-hold limit or exercise (Kooyman *et al.*, 1980; Williams *et al.*, 1993). Still, for Noren *et al.* (2012b) concluded that apnea of 13.3 min in an adult male killer whale did not caused a rise in blood lactate levels, it was assumed that lactate increase concerning breath-hold did not influence analyses outcomes since maximum apnea duration observed was 6.1 and 5.3 min for males and females, respectively. Nevertheless, it is hypothesized that anaerobic metabolism is valuable to include in the future O_2 model concerning short-term anaerobic sprints, for instance during hunting. Moreover, the potential of the established model lies in the fact that it is operational to study also the energetics and respiration of other cetacean species, if adjusted where needed. Extension concerning anaerobic metabolism and the dive response features would make it applicable to deep-diving

cetaceans.

Though the influence by CO_2 accumulation during apnea on respiration timing has been incorporated to diving models for aquatic birds, it has not been applied to models for marine mammals (Wilson *et al.*, 2003; Green *et al.*, 2005; Mortola and Seguin, 2009; Houston, 2011). Also, the removal of CO_2 through breathing will influence surface interval times, or the number of consecutive breaths, as much as O_2 replenishment (Boutilier *et al.*, 2001; Wilson *et al.*, 2003; Green *et al.*, 2005). To improve accuracy of energetic estimates using the established O_2 model, the inclusion of CO_2 is essential.

3. Stroking rate as an activity metric in contrast to speed

3.1. Introduction

For studies which applied Kramer's (1988) optimal diving model merely speed was taken into account (Thompson *et al.*, 1993; Thompson and Fedak, 2001; Acevedo-Gutiérrez *et al.*, 2002). However, according to the author's knowledge, no such study exists incorporating stroking. A possible explanation for this is that concrete measures of MR in relation to stroking activity are difficult to obtain for marine mammals (Meir *et al.*, 2013), especially for large species that cannot be held in captivity. Still, besides speed, stroking rate could be an adequate alternative activity metric to apply to the O_2 model for it is a direct reflection of animal effort and thus V_{O2} (Williams *et al.*, 2004a; Davis and Williams, 2012; Noren *et al.*, 2012a; Davis, 2014).

As producing a stroke entails a stereotyped motion by a swimming animal, it has been considered to be a strong predictor of swimming effort (Williams *et al.*, 2004a; Wilson *et al.*, 2006). Williams *et al*. (2004) correlated MR over entire dives with the number of strokes made by swimming Weddell seals to derive a metabolic 'cost per stroke'. Swimming strokes of killer whales are apparent in DTAG records. Thus, the aim of this part of the study was to evaluate how the established O_2 model presented in Chapter 2 would perform according to the implementation of stroking rate as a metric reflecting activity level in contrast to speed.

3.2. Material and methods

The same data set and general methods were applied as discussed in Chapter 2 of this thesis. Methods specific to this chapter are detailed below.

3.2.1. Detection of strokes

For this part of the study level of activity was assessed within individual tagging records through the number of fluke strokes made by the tagged whale per unit time. Stroking rates

were related to dorsal-ventral thrusting movements of each whale's flukes and body rotations associated with each thrusting stroke. Strokes were identified using the "*findflukes*"-function in MATLAB® (Johnson, unpublished data). To use this function several parameters had to be calculated beforehand. The minimal magnitude threshold in radians to detect a fluke stroke was derived from the pitch-rate signal frequency distribution, which in turn was derived from magnetometer data, and ranged between 0.0195 and 0.0394. The nominal stroking rate in Hz was derived from accelerations in pitch and counted between 0.3713 and 0.5472, as the lowest and highest values found, respectively. This rate is used to set the cut-off frequency of a low-pass filter to detect strokes through the "*findflukes*"-function. Both the magnitude threshold and the nominal stroking rate were determined for every individual separately. The minimum and maximum duration allowable for a fluke stroke of 0.4 and 5.0 s, respectively, was specified. Using this code a fluke stroke was counted whenever there was a cyclic variation in the pitch deviation with peak-to-peak magnitude larger than ± the threshold and coinciding with duration between the minimum and maximum allowed duration (Johnson and Tyack, 2003). This method was manually tested through counting stroking rates from pitch data with the naked eye over certain time intervals selected randomly for various individuals. Through this approach each fluke stroke was detected within the whale frame accelerometer data record.

Individual time series data of stroking rate were investigated for outliers, or inaccuracies, potentially caused by small tag sensor errors. Stroking rate measurements for 1s intervals which were higher than the first quartile plus three times the interquartile range (IQR) were considered as unrealistically high and therefore replaced with the mean stroking rate for that specific record. Depending on the variance of stroking rate measurements within an individual the 'size' of outliers could vary between whales, for the IQR was relative to this variance. Nonetheless, swimming movements recorded by the tag, and therefore also stroking, are unique per whale due to variation in tag placement on the body.

3.2.2. Metabolic cost in relation to stroking

The slope of MRL vs. speed^3 was adjusted to reflect estimated MR_L according to stroking rate per gender, assuming that each fluke stroke entails an identical metabolic cost (Williams *et al.*, 2004a). Kriete (1995) measured MR directly for different states of activity, but did not link

these states to stroking rate. Because there were no known relationships for O_2 use related to stroking activity in killer whales at the time of the present study, values for coefficient *k* were estimated indirectly from how MR was estimated from speed (Section 2.2.5). High-activity values were estimated from the linear correlation between stroking rate versus speed per gender. The MR estimated according to speed was related back to stroking rate using the results of the linear correlation (see Fig. 4.2). Subsequently, a predicted relationship between MR and stroking rate was founded per sex (Fig. 3.1).

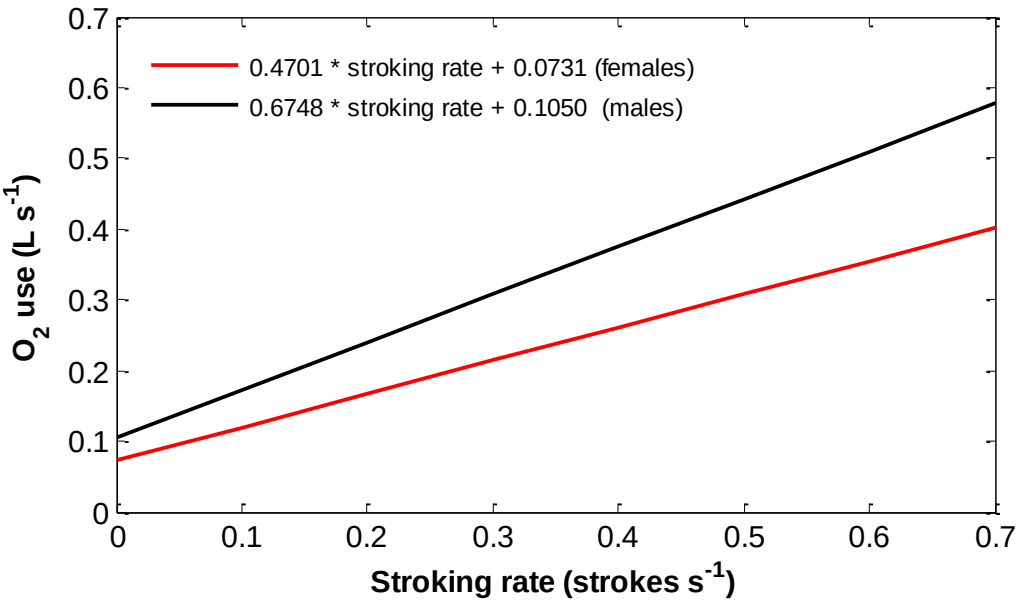

Figure 3.1. O_2 use as a function of stroking rate (including basal metabolic rate) for adult female and male killer whales as implemented in the O_2 model. Males were expected to have a higher cost per fluke stroke in comparison to females due to a bigger body mass.

3.2.3. The O_2 model

The O_2 model including the different E_{O2} per breath as explained in Section 2.2.6 was now executed with stroking rate measurements as the activity metric used to estimate MR per 15 min intervals (Section 2.2.7). All other parameters remained equal apart from the slope of MR_L vs. stroking rate (*k*). Consequently, MR estimates per 15 min intervals were plotted against stroking rate measurements per 15 min.

3.2.4. Statistical modelling and analyses

The influence of V_{O2} on respiration rate and E_{O2} over the 15 min blocks was analysed using the same methods as explained in Section 2.2.8, here using stroking rate as explanatory variable. Again, it was hypothesized that V_{02}, and therefore level of activity, and E_{O2} should be balanced over the 15 min blocks for short-duration diving in the examined whales, showing a positive linear correlation as each stroke was assumed to have an identical metabolic cost.

As for the speed measurements, data analyses were conducted on the stroking rate data to select the appropriate statistical model. All test results were identical as for speed except for the fact that for stroking data none of these tests indicated substantial auto-correlation within any of the individuals. Therefore a GLM was applied to model estimated MR versus stroking rate over 15 min intervals per individual. Still, a same GEEGLM was applied to account for random effects by individuals for analyses per sex category as explained in Section 2.2.8.1. For evaluation of goodness of fit of the models see Section 2.2.8.2.

3.2.5. Sensitivity analyses

Sensitivity analyses regarding BMR and maximum E_{O2} were executed as explained in Section 2.2.9.2 and 2.2.9.3, respectively.

3.2.5.1. Sensitivity rates for metabolic costs of strokes

Because accurate information on the O_2 use per fluke stroke for killer whales was lacking altogether, these were estimated from extrapolating MR information on speed (Guinet *et al.*, 2007) and so the deviation from the used value to its true value was uncertain. Therefore, the slope of MR_L vs. stroking rate (*k*) required to estimate MR_L potentially contained inaccuracies which could potentially influence the model outcomes.

To the author's knowledge, the only information on O_2 cost per stroke for a marine mammal was collected on Weddell seals by (Williams *et al.*, 2004a), who estimated that for one stroke an adult Weddell seal would use 0.044 ml $O_2 \cdot kg^{-1}$. According to this value an adult male killer

whale of 3,913 kg and an adult female killer whale of 2,800 kg would use 0.0963 and 0.0749 LO_2 per stroke, respectively, which is 7.0 and 6.3 times smaller than values used during the present study. These values were set as the lower range values for the sensitivity analyses concerning the slope of MR_L vs. stroking rate. There was no literature available on a possible maximum limit of O_2 use per stroke. Therefore, the higher limit was set by adding the difference between the used values (Fig. 3.1) and the values used to set the lower limit per gender.

3.3 Results

3.3.1. Stroking rate measurements

For all killer whales together a minimum, maximum and average (± standard deviation) stroking rate per second over a 15 min interval was found of 0.062, 0.507, respectively, and 0.307 (± 0.0805) (Table 3.1). The maximum stroking rate over 15 min was observed for one of the males (Table 3.1). Both sexes exhibited periods without stroking, which could indicate "gliding" if the animal still showed forward movement. The difference between the mean stroking rate measured for females (0.292 ± 0.1056, Table 3.1) and males (0.314 ± 0.0644) was not significant (Wald-test, $P = 0.520$).

All individuals exhibited multiple outliers (Appendix 3.1 and 3.2) which were replaced with mean stroking rates for particular tagging records before running analyses. However, the outliers were uncommon (Appendix 3.2) and always short in duration, and their replacement should not influence overall patterns.

3.3.2. O_2 use versus activity for different O_2 models

3.3.2.1. The O_2 model using fixed O_2 uptake per breath

For all adult killer whales together, only 14.7 % of breathing rate variability could be explained by stroking rate (Table 3.2). Similar low correlation strength was found for females, whereas

for males there appeared to be no correlation at all between breathing rate and stroking rate (Table 3.2). Slope of the simple linear regression was the highest for males. However, due to the lack of statistical correlation between breathing rate and stroking this slope is assumed to be non-informative. The slope for the female model was minimal (Table 3.2, Fig. 3.2), showing that breathing rate did not increase significantly according to stroking rate. There was weak statistical evidence that respiration rate related to any stroking rate was higher for males than for females (Wald-test, Wald-value = 4.58, $P = 0.0324$).

Table 3.1. Average, minimum and maximum stroking rate measurements (strokes·s^{-1} over 15 min intervals) per individual killer whale, sex category and for all whales together.

	Whale ID	Mean stroking rate·s^{-1} (± SD)	Min stroking rate·s^{-1}	Max stroking rate·s^{-1}
Female	05_316a	0.231 (± 0.1156)	0.090	0.374
	05_320a	0.202 (± 0.0587)	0.096	0.290
	05_321b	0.228 (± 0.0440)	0.183	0.275
	05_322a	0.310 (± 0.1098)	0.131	0.456
	06_313s	0.300 (± 0.0669)	0.218	0.402
	06_327s	0.391 (± 0.0531)	0.268	0.441
	All females	**0.292 (± 0.1056)**	**0.090**	**0.456**
Male	05_320b	0.250 (± 0.0518)	0.209	0.331
	05_322b	0.244 (± 0.1086)	0.062	0.334
	06_314a	0.377 (± 0.0345)	0.314	0.409
	06_327t	0.273 (± 0.0350)	0.189	0.315
	09_144a	0.337 (± 0.0458)	0.235	0.488
	09_144b	0.324 (± 0.0600)	0.220	0.507
	All males	**0.314 (± 0.0644)**	**0.062**	**0.507**
	Overall	**0.307 (± 0.0805)**	**0.062**	**0.507**

Table 3.2. Regression statistics and relationship values between stroking rate and respiration rate per sex category and for all killer whales together.

	# 15 min blocks	r^2	RMSE	Intercept	Slope
Females	63	0.171	0.0034	0.0212	0.0074
Males	131	0.042	0.0037	0.0188	0.0238
All	194	0.147	0.0037	0.0193	0.0186

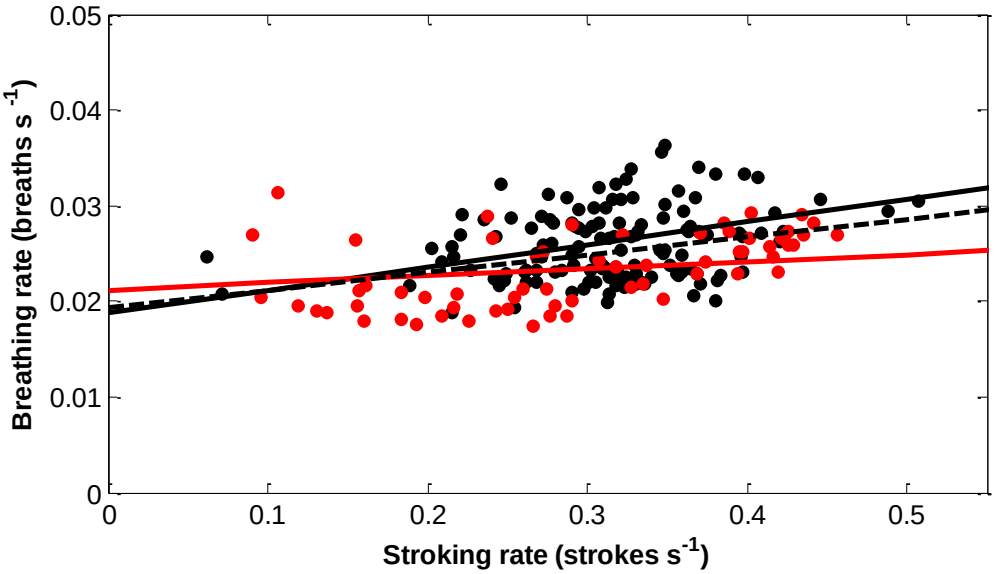

Figure 3.2. Scatter plot of breathing rate averaged over 15 min blocks versus stroking rate averaged over 15 min blocks. Solid lines represent the fitted model for females (red) and males (black). The dashed line represents fitted model for all individuals together.

As E_{O2} under this model was estimated by multiplying respiration rate by a constant value and adding the BMR, the same poor correlations were found between predicted E_{O2} and stroking activity over 15 min periods per sex category. Estimated MR for males was considerably higher than the BMR, which caused the coefficient of the model with the intercept set at BMR to be relatively steep, while model fit was negative, meaning that the model fitted the data worse than a horizontal line at the mean E_{O2} (the null hypothesis) (Table 3.3, Fig. 3.3). Also, the variance in MR estimates for any stroking activity was higher for males than for females (Fig. 3.3). For all females together, correlation had an even larger negative r^2; this model did not cross any of the data points, potentially due to individual influence (Table 3.3, Fig. 3.3). Setting the intercept at BMR according to gender resulted in a negative r^2 value for all of the females and four of the males, while two of the males showed a relatively higher r^2 (Table 3.3).

Model fit was considerably higher for all individuals when not setting the intercept, with two of the females and two of the males having an r^2 higher than 0.5 (Table 3.3). The slope coefficient showed a rather large range between individuals (Table 3.3). Moreover, for two females there existed a negative relationship between E_{O2} and stroking rate, of which one showed a moderate correlation (Table 3.3). Almost all intercepts found through the O_2 model with fixed E_{O2} without setting an intercept were substantially higher than the BMR found by Kriete (1995) of 0.0731 or 0.105 for females and males, respectively (Table 3.3). Only for one of the males (06_314a) was the intercept was below the estimated BMR (Table 3.3).

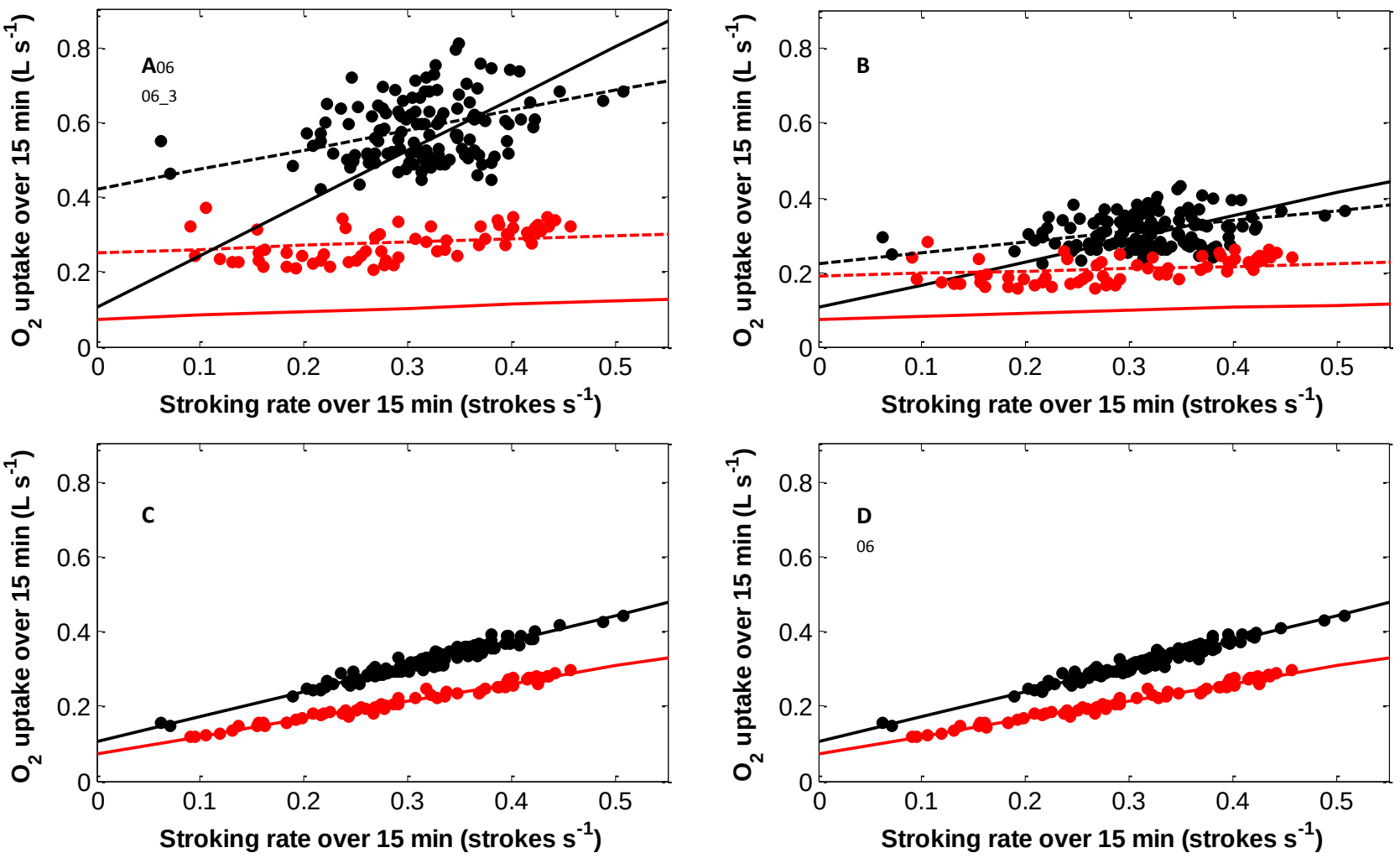

Figure 3.3. O_2 uptake versus stroking rate, both average over 15 min intervals, estimated through the O_2 model with fixed O_2 uptake(**A**), optimized fixed O_2 uptake (**B**), fluctuating O_2 uptake according to a set O_2 uptake curve (**C**), and fluctuating O_2 uptake according to an optimized O_2 uptake curve (**D**) for males (black) and females (red). Solid lines represent the O_2 model fitted with intercept set at BMR, dashed lines represent the O_2 model without set intercept.

Table 3.3. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model including a fixed uptake per breath (with and without set intercept at BMR), versus level of activity represented by stroking rate over 15 min intervals for individuals and sex categories.

		Intercept set at BMR			No set intercept			
Female	**# 15 min blocks**	**r^2***	**RMSE**	**Slope**	**r^2**	**RMSE**	**Intercept**	**Slope**
05_316a	7	-12.675	0.1248	0.7884	0.663	0.0196	0.3664	-0.2568
05_320a	16	-8.730	0.0472	0.7192	0.022	0.0150	0.2387	-0.0394
05_321b	4	-44.398	0.0278	0.7460	0.094	0.0039	0.2400	0.0332
05_322a	11	-0.085	0.0370	0.5513	0.655	0.0209	0.1686	0.2747
06_313s	6	-0.515	0.0500	0.7620	0.129	0.0379	0.2364	0.2388
06_327s	19	-1.210	0.0268	0.6051	0.278	0.0153	0.2406	0.1837
All females	**63**	**-15.541**	**0.1784**	**0.1000**	**0.171**	**0.0399**	**0.2523**	**0.0877**
Male								
05_320b	5	-3.872	0.0893	1.8569	0.034	0.0398	0.5431	0.1610
05_322b	9	-1.374	0.1565	1.8050	0.364	0.0810	0.4514	0.5987
06_314a	6	0.584	0.0334	1.1451	0.589	0.0332	0.0607	1.2618
06_327t	20	0.452	0.0402	1.8847	0.614	0.0338	0.2811	1.2493
09_144a	44	-0.149	0.0660	1.3688	0.186	0.0556	0.3727	0.5878
09_144b	47	-0.007	0.1055	1.4130	0.158	0.0965	0.3419	0.7051
All males	**131**	**-0.751**	**0.1110**	**1.3939**	**0.042**	**0.0821**	**0.4197**	**0.5318**

* A negative r^2-value indicates that the fit of the model is worse than the fit of a horizontal line at the mean y-value (null hypothesis).

Applying the O_2 model with fixed E_{O2}, as employed by Williams and Noren (2009), resulted in an unrestrained and unrealistically high O_2 store caused by an imbalance of the O_2 use and uptake over the tag period for all individuals (Fig. 3.4, Appendix 3.3). In reality the O_2 store is not expected to exceed the maximum capacity of the animal which was estimated to be 137.3 and 99.9 L for an adult male and female killer whale, respectively (Noren *et al.*, 2012b).

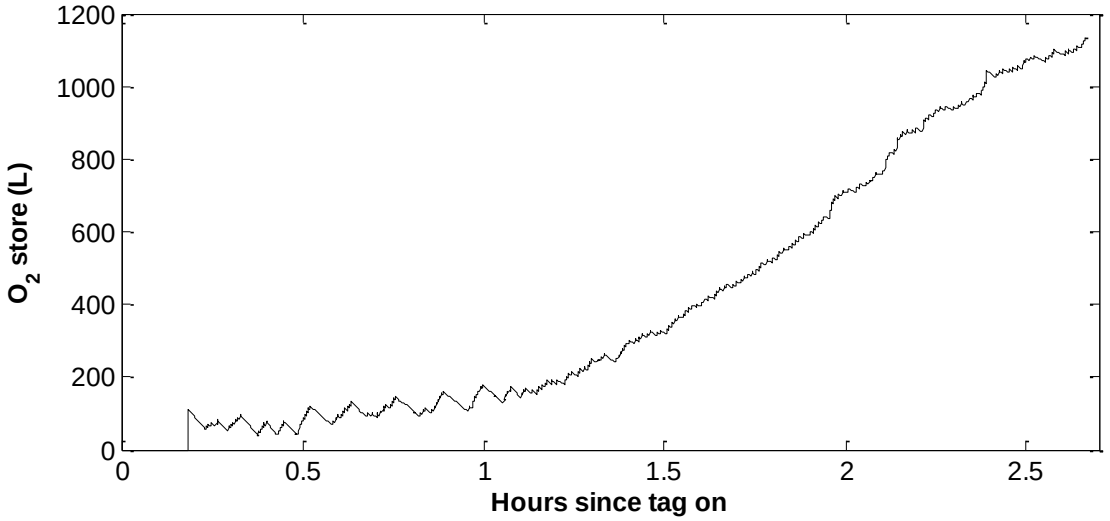

Figure 3.4. Example of a time series plot of the O_2 store for female 05_316a estimated over the entire tag recored through the O_2 model using the fixed O_2 uptake per breath as used in existing literature, including Williams and Noren (2009).

3.3.2.2. The O_2 model using optimized fixed O_2 uptake

The optimized fixed E_{O2} ranged from 7.39 and 10.41 L for females and 9.53 to 14.96 L for males (Table 3.4). The males had on average a significantly (*t*-Test, $P = 0.00531$) higher optimized fixed E_{O2} per breath (11.90 L, N = 6, SD = 2.028) than the females (8.93 L, N = 6, SD = 1.139) (Table 3.4). For all females, expect one, it appeared there was no relationship whatsoever between stroking and E_{O2} over the 15 min intervals (negative r^2, Table 3.4) when setting the intercept of at the BMR. For the one female of which the r^2 value was positive, the relationship strength between stroking and E_{O2} was negligible (Table 3.4). The results for the males were rather diverse: for two of the males there was a reasonable strong correlation between stroking and the E_{O2} over the 15 min intervals, while for two others there was almost no correlation, and again two others there was no correlation at all (Table 3.4). Applying the optimized fixed E_{O2} per breath averaged either for all females or all males to the O_2 model with the intercept set at BMR, resulted in no relationship between stroking and estimated E_{O2} over the 15 min intervals (Table 3.4). Model slopes of all individuals was high, due to the set intercept at BMR which is apparently substantially lower than when the intercept was undefined, as was also shown when applying the non-optimized fixed E_{O2} (Fig. 3.3). When applying the model for all females together, taking into account random effects by the individuals, the coefficient was so off that the model was not following the data trend whatsoever (Table 3.4, Fig. 3.3).

Table 3.4. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model including an optimized fixed uptake per breath (with intercept set at BMR), versus level of activity represented by stroking rate over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized fixed E_{O2} (L)	r^{2*}	RMSE	Slope
Female	05_316a	7	7.39	-8.927	0.0660	0.3909
	05_320a	16	9.42	-6.814	0.0335	0.4995
	05_321b	4	8.67	-31.063	0.0170	0.4588
	05_322a	11	10.41	0.068	0.0300	0.4557
	06_313s	6	7.94	-0.260	0.0304	0.4305
	06_327s	19	9.73	-0.935	0.0205	0.4612
		Average	**8.93**	**-11.262**	**0.1153**	**0.0781**
Male	05_320b	5	10.97	-2.177	0.0355	0.7069
	05_322b	9	9.53	-0.250	0.0486	0.5625
	06_314a	6	14.96	0.565	0.0229	0.6772
	06_327t	20	10.23	0.600	0.0158	0.6595
	09_144a	44	13.06	0.012	0.0359	0.6747
	09_144b	47	12.67	0.086	0.0572	0.6673
		Average	**11.90**	**-0.362**	**0.0522**	**0.6163**

* A negative r^2-value indicates that the fit of the model is worse than the fit of a horizontal line at the mean y-value (null hypothesis).

When applying the O_2 model with optimized E_{O2} without setting the intercept, the model fit improved substantially for all individuals and bot sex categories and there were no negative r^2 values (Table 3.5, Fig. 3.3). Still, correlation between MR estimates and activity level was poor for both categories and most individuals with only two females and one male exhibiting a r^2 value higher than 0.5 (Table 3.5). Moreover, because MR was estimated through multiplying breathing rate with a fixed E_{O2} value, correlations between estimated MR and stroking were the same as for using the non-optimized fixed E_{O2} without setting the model intercept. The model for the males as category had a worse fit for most of the males individually (Table 3.5). The model coefficient for males was somewhat higher than for females (Table 3.5, Fig. 3.3).

Table 3.5. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model including an optimized fixed uptake per breath (without set intercept), versus level of activity represented by stroking rate over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized fixed E_{O2} (L)	r^2	RMSE	Slope	Intercept
Female	05_316a	7	7.39	0.663	0.0147	-0.1927	0.2750
	05_320a	16	9.42	0.022	0.0112	-0.0296	0.1791
	05_321b	4	8.67	0.094	0.0029	0.0242	0.1749
	05_322a	11	10.41	0.655	0.0183	0.2403	0.1475
	06_313s	6	7.94	0.129	0.0253	0.1593	0.1578
	06_327s	19	9.73	0.278	0.0125	0.1502	0.1968
	Average		**8.93**	**0.171**	**0.0230**	**0.0658**	**0.1893**
Male	05_320b	5	10.97	0.034	0.0196	0.0792	0.2672
	05_322b	9	9.53	0.364	0.0346	0.2559	0.1929
	06_314a	6	14.96	0.415	0.0255	0.4294	0.2015
	06_327t	20	10.23	0.614	0.0155	0.5731	0.1289
	09_144a	44	13.06	0.186	0.0325	0.3442	0.2183
	09_144b	47	12.67	0.158	0.0548	0.4006	0.1942
	Average		**11.90**	**0.042**	**0.0438**	**0.2838**	**0.2240**

Applying the fixed E_{O2} per breath optimized per individual to the tag records resulted in the unrealistically event of excess of the estimated TBO for six of the females and five of the males (Fig. 3.5, Appendix 3.3). Also, for two males and four females O_2 store became negative (Appendix 3.3). Besides, the O_2 store was not balanced throughout the tag record for most of the individuals, meaning that there was no balance between the O_2 use and O_2 uptake over periods shorter than the tag record duration (Fig. 3.5, Appendix 3.3) as reflected by the low r^2 values in Table 3.5. These results indicated an overall poor correlation between estimated E_{O2} and activity level over 15 min intervals.

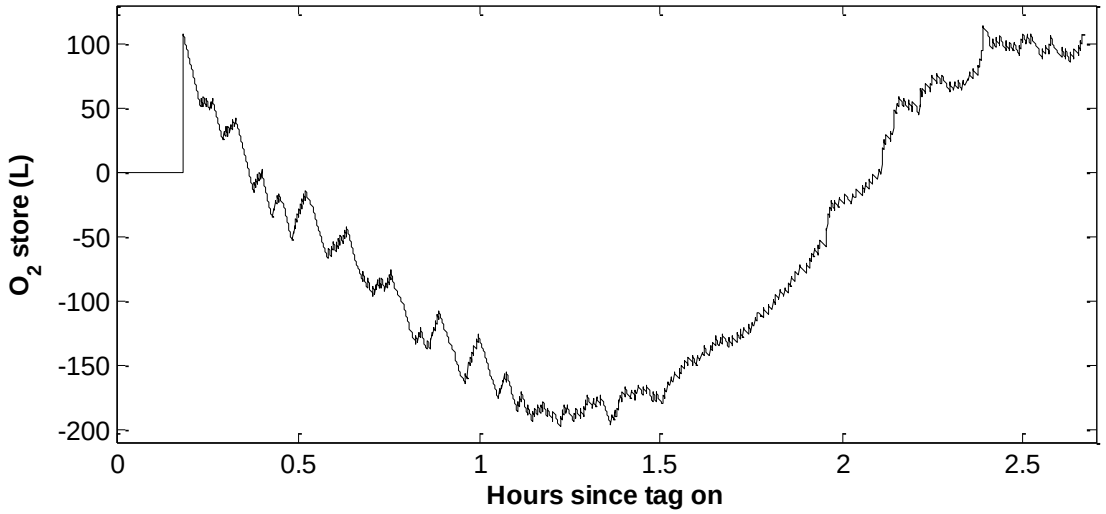

Figure 3.5. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 3.4) estimated over the entire tag recored through the O_2 model using optimized fixed O_2 uptake per breath.

3.3.2.3. The O_2 model including a set O_2 uptake curve

Applying a set O_2 uptake curve to the O_2 model resulted in strong correlations between stroking rate and estimates E_{O2} over the 15 min blocks for all indvivuals and categories (Table 3.6, Fig. 3.3). Correlation strength ranged between 0.886 (male 06_314a) and 0.982 (female 05_322a) (Table 3.6). Obviously, as E_{O2} per breath for this model depended on O_2 store, which in turn was shaped by the level of activity, plus the BMR was extracted from the estimated E_{O2} to model only locomotion costs, the model coefficient approximated the the slope of MR_L vs. stroking rate (Fig. 3.1) for all individuals (Table 3.6). The biggest deviation of the slope of MR_L vs. stroking rate was found for female 05_316a, which was 0.0129 $LO_2 \cdot stroke^{-1} \cdot s^{-1}$.

For this O_2 model, the O_2 store was balanced throughout the tag record for all individuals and did not exceed the estimated TBO capacity: when the animals took many breaths in a row after a longer dive the first couple of breaths were sufficient to replenish the store. However as the TBO became more saturated, E_{O2} per breath was less efficient (Fig. 3.6, Appendix 3.3). Also, the O_2 store was not depleted completely for any of the individuals under this model.

Table 3.6. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model including a set O_2 uptake curve (with intercept set at BMR), versus level of activity represented by stroking rate over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	r^2	RMSE	Slope
Female	05_316a	7	0.976	0.0082	0.4829
	05_320a	16	0.903	0.0081	0.4642
	05_321b	4	0.975	0.0032	0.4757
	05_322a	11	0.982	0.0069	0.4762
	06_313s	6	0.959	0.0067	0.4769
	06_327s	19	0.942	0.0062	0.4681
	All females	**63**	**0.979**	**0.0072**	**0.4690**
Male	05_320b	5	0.955	0.0066	0.6791
	05_322b	9	0.970	0.0123	0.6805
	06_314a	6	0.886	0.0068	0.6735
	06_327t	20	0.956	0.0051	0.6746
	09_144a	44	0.889	0.0104	0.6745
	09_144b	47	0.931	0.0111	0.6754
	All males	**131**	**0.950**	**0.0099**	**0.6749**

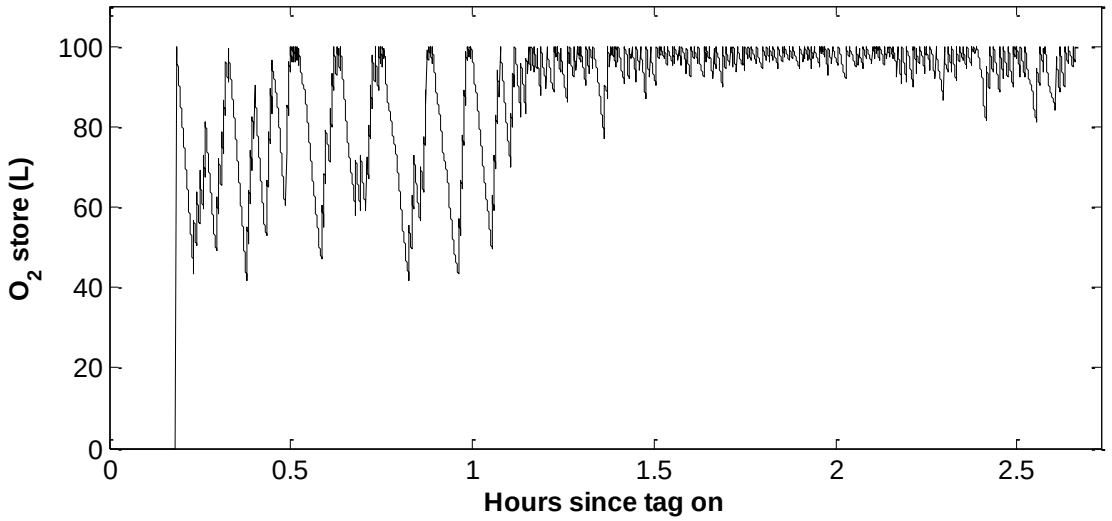

Figure 3.6. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 3.4) estimated over the entire tag recored through the O_2 model including a set O_2 uptake curve.

3.3.2.4. The O_2 model including an optimized O_2 uptake curve

The optimized exponent for the density dependent O_2 uptake curve showed a rather large range between 4.12 to 21.64 and 2.42 to 9.40 for females and males, respectively (Table 3.7, Fig. 3.7). However, for four of the females, one of the males, and females as category there existed a horizontal asymptote when plotting the r^2 values against the exponent values as was found when using speed as an activity metric (Section 2.3.3.4) Again, it was concluded that for these five individuals and females as category the model including the non-optimized uptake curve fitted the data better than a density dependent curve. As explained in Section 2.3.3.4, an approximation of optimized exponent value was conducted for individuals showing an asymptote. The relationship between stroking rate and estimated E_{O2} over the 15 min intervals

Table 3.7. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using an optimized O_2 uptake curve (with intercept set at BMR), versus level of activity represented by stroking rate over 15 min intervals for individuals and sex categories.

	Whale ID	# 15 min blocks	Optimized exponent	r^2	RMSE	Slope
Female	05_316a	7	4.12	0.988	0.0058	0.4891
	05_320a	16	(21.64)*	(0.902)	(0.0082)	(0.4642)
	05_321b	4	(12.24)*	(0.974)	(0.0033)	(0.4758)
	05_322a	11	(17.71)*	(0.981)	(0.0069)	(0.4764)
	06_313s	6	(17.21)*	(0.958)	(0.0068)	(0.4759)
	06_327s	19	6.59	0.948	0.0058	0.4680
	All females	**63**	**18.58***	**0.979**	**0.0073**	**0.4690**
Male	05_320b	5	(9.40)*	(0.954)	(0.0066)	(0.6790)
	05_322b	9	8.19	0.970	0.0122	0.6806
	06_314a	6	2.68	0.926	0.0060	0.6760
	06_327t	20	2.85	0.968	0.0043	0.6745
	09_144a	44	2.42	0.916	0.0089	0.6738
	09_144b	47	4.25	0.935	0.0107	0.6753
	All males	**131**	**3.77**	**0.954**	**0.0095**	**0.6745**
	Overall	**194**	**9.53**	**0.687**	**0.0324**	**0.5852**
	Females			0.976	0.0077	0.4691
	Males			0.950	0.0099	0.6749

* No automatic optimization possible due to the presence of asymptote, see text for further details.

was considerably strong for all individuals and for females and males as categories. The model with an optimized O_2 uptake curve for all whales together (Fig. 3.8) resulted in a less strong correlation between activity level and estimated E_{O2} than the correlations within sex categories (Table 3.7). Applying this same optimized exponent for the sex categories separately increased the fit of the model, resulting in r^2 values just a bit lower than r^2 values found for the model including the curve exponents optimized per sex category (Table 3.7). Model coefficients approached the slope of MR_L vs. stroking rate for all individuals and both sex categories. The coefficient of the model combining both sexes lies in between as expected (Table 3.7).

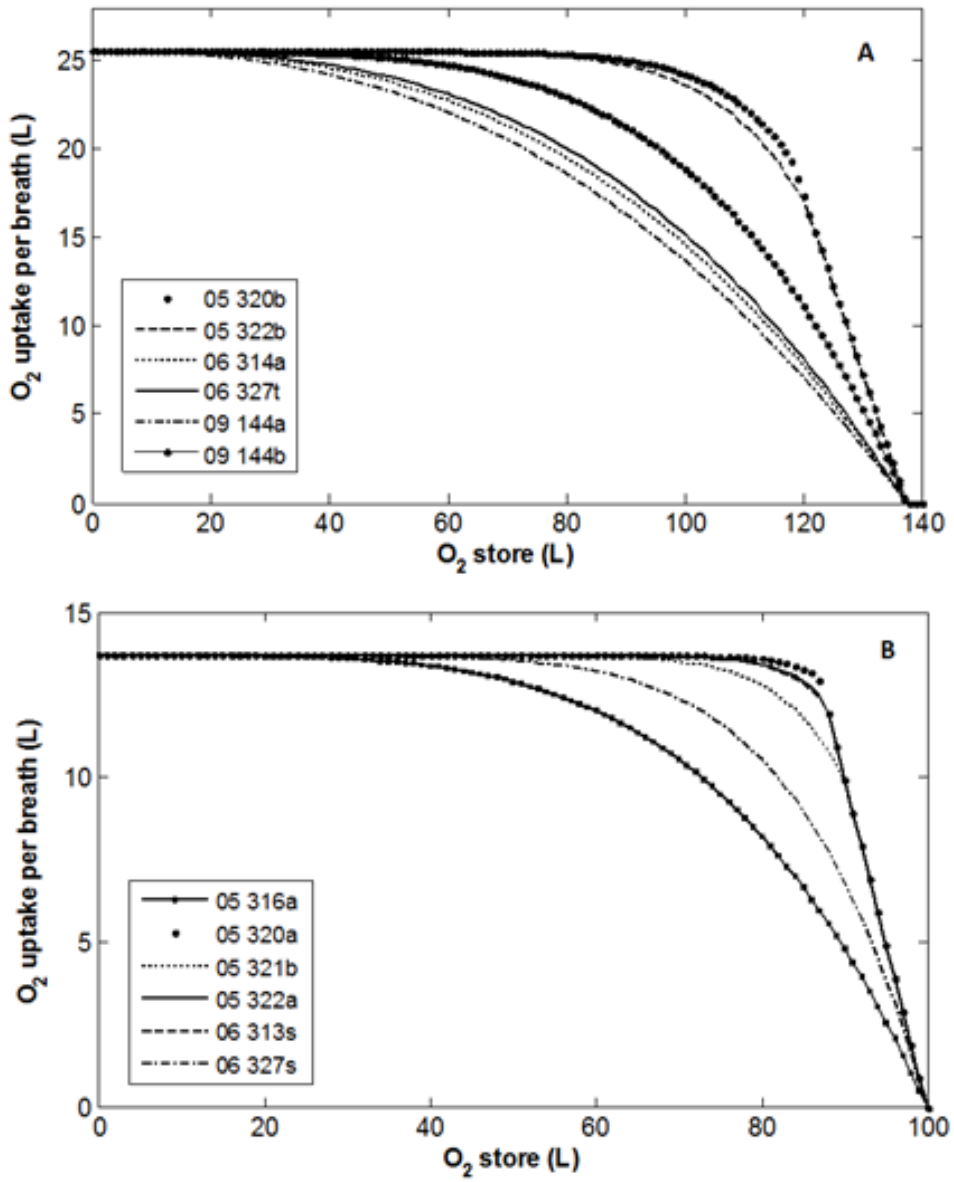

Figure 3.7. The O_2 uptake per breath as a function of the O_2 store at the time of each breath taking the form of a density dependent curve, shaped by the exponent optimized per individual male (A) and female (B).

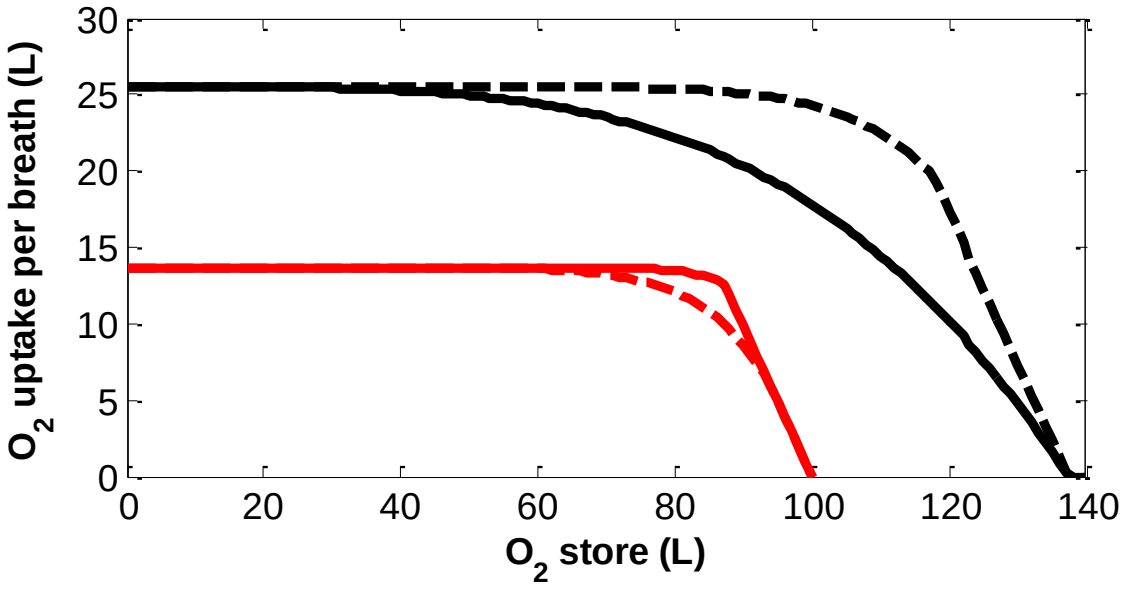

Figure 3.8. The O_2 uptake as a function of the O_2 store at the time of each breath taking the form of a density dependent curve, shaped by the exponent optimized for males (black solid line), females (red solid line), and all killer whales together applied to males (black dotted line) and females (red dotted line).

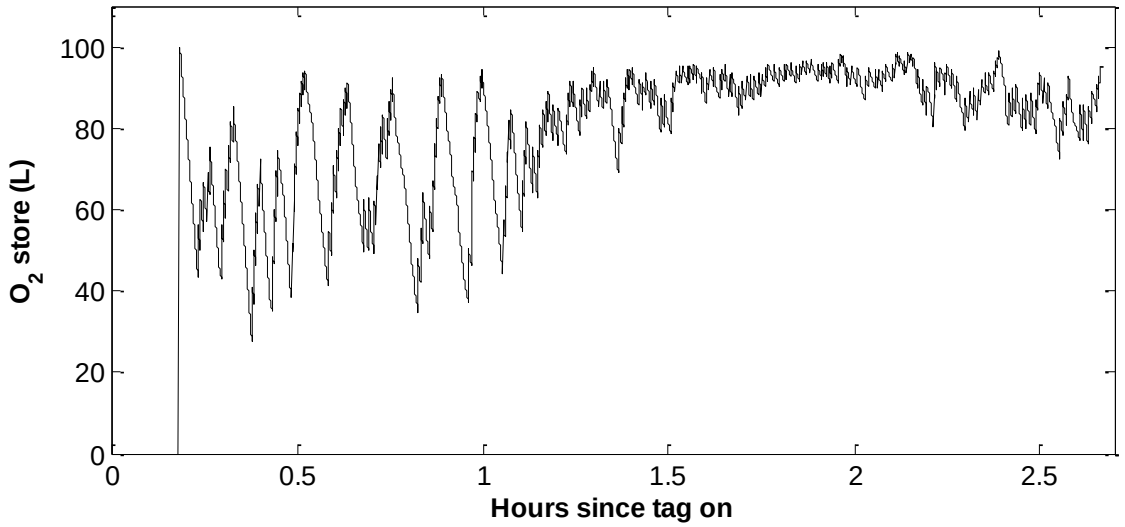

Figure 3.9. Example of a time series plot of the O_2 store for female 05_316a (same individual as Fig. 3.4) estimated over the entire tag recored through the O_2 model including its optimized O_2 uptake curve.

The O_2 store estimated throughout the tagging records for all individuals applying the O_2 model with optimized O_2 uptake curve was more balanced, without exceeding the TBO capacity or depleting to unrealistically low levels (Fig. 3.9). Therefore it is expected that using this model the E_{O2} estimates, which show a tight correlation with the activity level, are valuable.

3.3.3 Sensitivity analyses

3.3.3.1. Metabolic Rates

All slopes of MR_L vs. stroking rate (*k*) applied for the sensitivity analyses produced high, though somewhat lower r^2 values compared to the used value for males (Table 3.8). By increasing the slope of MR_L vs. stroking rate for males by 85.7 % the model fit decreased by 5.4 %, while by decreasing the slope of MR_L vs. stroking rate by the same percentage the model fit decreased by 16.8 % (Table 3.8). Understandably, for both males and females the model residual errors decreased with a decreasing slope of MR_L vs. stroking rate (Table 3.8, Fig. 3.11). The model fit regarding the females decreased by 70.9 % when the slope of MR_L vs. stroking rate was increased by 84.0 % (Table 3.8); for at least some females this highest slope of MR_L vs. stroking rate resulted in very poor correlation between estimated E_{O2} and stroking (Fig. 3.11). However, by decreasing the slope of MR_L vs. stroking rate by 41.9 % the model fit increased by 0.3 % compared to the model fit relative to the value used (Table 3.8).

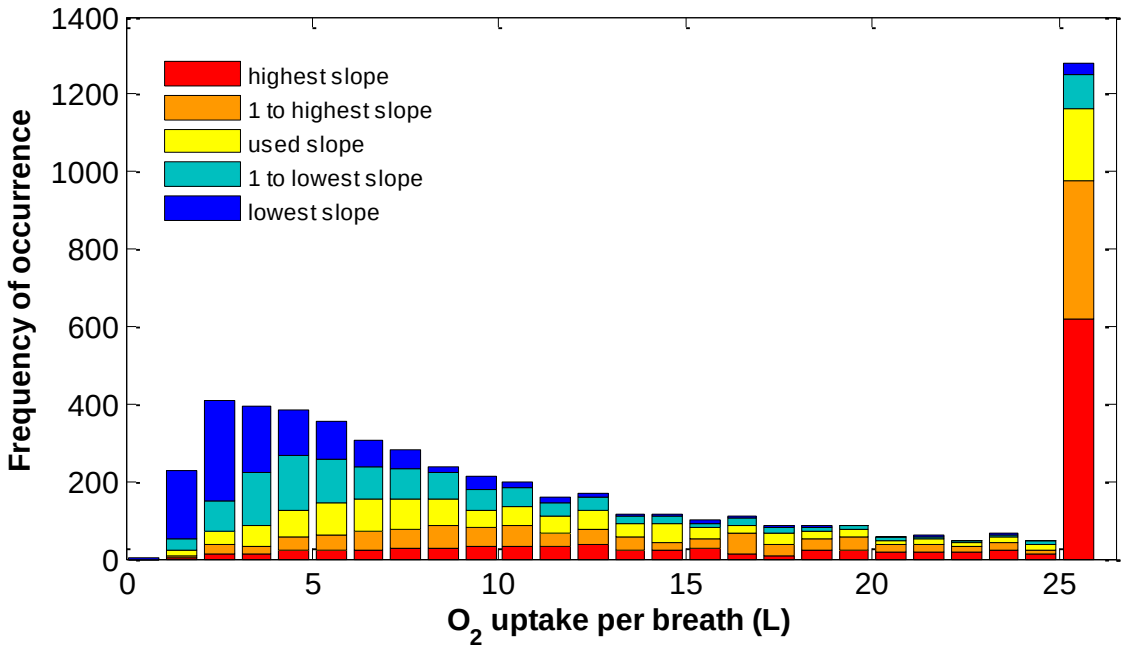

Figure 3.10. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for the slope of MR_L vs. stroking rate (*k*) incorporated into the O_2 model with a set O_2 uptake curve.

Table 3.8. Regression statistics and relationships between O_2 uptake estimated over the 15 min intervals with the O_2 model using a set O_2 uptake curve (with intercept set at BMR), versus level of activity represented by stroking rate over 15 min intervals, according to different slopes of MR_L vs. stroking rate, basal metabolic rates, and maximum O_2 uptake per breath per sex category.

Males	**Slope of MR_L vs. stroking rate (*k*)**	r^2	**RMSE**	**Slope**
(131 blocks)	**1.253**	0.899	0.0271	1.2518
	0.964	0.937	0.0160	0.9639
	0.386	0.943	0.0061	0.3862
	0.096	0.790	0.0031	0.0961
	Basal metabolic rate	r^2	**RMSE**	**Slope**
	0.281	0.804	0.0217	0.6737
	0.193	0.898	0.0146	0.6747
	0.098	0.953	0.0096	0.6749
	0.090	0.956	0.0093	0.6750
	Max. O_2 uptake per breath	r^2	**RMSE**	**Slope**
	54.16	0.968	0.0079	0.6751
	39.84	0.963	0.0085	0.6751
	22.29	0.942	0.0107	0.6749
	19.06	0.921	0.0127	0.6746
Females	**Slope of MR_L vs. stroking rate (*k*)**	r^2	**RMSE**	**Slope**
(63 blocks)	**0.865**	0.285	0.0493	0.7213
	0.668	0.879	0.0234	0.6652
	0.273	0.982	0.0038	0.2715
	0.075	0.945	0.0019	0.0743
	BMR	r^2	**RMSE**	**Slope**
	0.197	0.385	0.0368	0.3540
	0.135	0.895	0.0163	0.4697
	0.072	0.979	0.0072	0.4689
	0.070	0.980	0.0070	0.4689
	Max. O_2 uptake per breath	r^2	**RMSE**	**Slope**
	31.22	0.991	0.0048	0.4681
	22.45	0.989	0.0052	0.4680
	12.06	0.963	0.0099	0.4735
	10.43	0.850	0.0183	0.4669

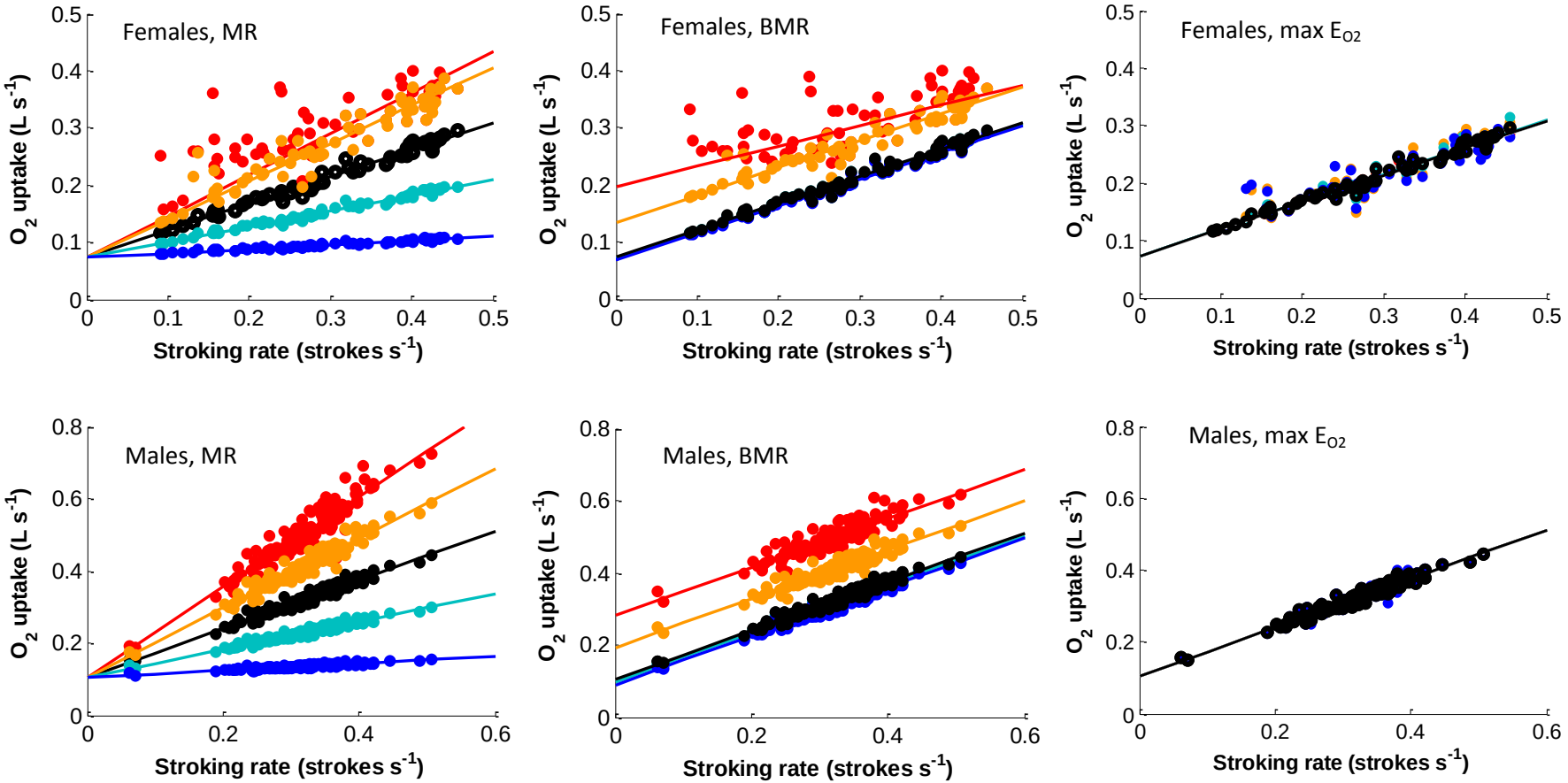

Figure 3.11. O_2 uptake per 15 min intervals as estimated by the O_2 model including a set O_2 uptake curve versus stroking rate per sex category, with different values for the slope of MR_L vs. stroking rate (*k*) (MR), basal metabolic rate (BMR) and maximum O_2 uptake per breath E_{O2}) implemented for sensitivity analyses of the model, where red represents highest, orange one to highest, aqua one to lowest, and blue lowest values. The original model outcomes are coloured black.

The lowest slope of MR_L vs. stroking rate assessed during the sensitivity analyses for the model using the set O_2 uptake curve resulted in a decrease in overall O_2 uptake per breath (Fig. 3.10). This was caused by a relatively low O_2 use and subsequently a smaller decrease in O_2 store during breath intervals compared to the implementation of the used slope of MR_L vs. stroking rate. As the O_2 use derived through this slope of MR_L vs. stroking rate was rather low, the animal could fully replenish the O_2 store during a single breath on many occasions; the frequency of breaths during which the animal would took up maximal possible O_2 were few. On the contrary, when implementing the highest slope of MR_L vs. stroking rate, the O_2 store was expected to be depleted heavily during many apneas, and therefore the animal would need to take up O_2 maximally during most breathing events to replenish the store (Fig. 3.10). The slope of MR_L vs. stroking rate originally used in the model including a set O_2 uptake curve resulted in the most equal distribution of E_{O2} per breath less than the maximum E_{O2}, and an average occurrence of this maximum O_2 uptake (Fig. 3.10).

Slopes of MR_L vs. stroking rate optimized according to the fixed E_{O2} per breath value used by Kriete (1995) and Williams and Noren (2009) (see Section 2.3.4.1) were 1.1034 and 2.0067 for females and males, respectively, which was 2.3 and 3.0 times higher than the values used (Fig. 3.1). Applying these slopes of MR_L vs. stroking rate to the model with a set O_2 uptake curve did not result in a correlation between MR estimates and activity level for either females or males (Table 3.9). Slopes of MR_L vs. stroking rate optimized according to the optimized fixed E_{O2} per breath were 0.7323 and 0.8664 for females and males, being 55.8 % and 28.4 % higher, respectively, than the values used. These slopes of MR_L vs. stroking rate produced significantly tight correlations when implementing them in the O_2 model with O_2 uptake curve, yet model fit was not as high as according to the values used (Table 3.9).

Table 3.9. Regression statistics for O_2 uptake estimated over the 15 min intervals with the O_2 model using a set O_2 uptake curve and optimized slope of MR_L vs. stroking rate (*k*) according to the O_2 model with fixed O_2 uptake and optimized fixed O_2 uptake per breath, versus level of activity represented by stroking rate over 15 min intervals per sex category. Intercept was set at BMR.

	Slope of MR_L vs. stroking rate (*k*) (fixed E_{O2})				**Slope of MR_L vs. stroking rate (*k*) (opt. fixed E_{O2})**			
	k	***r*²**	**RMSE**	**Slope**	***k***	***r*²**	**RMSE**	**Slope**
Females	1.1034	-13.520	0.2029	0.1537	0.7323	0.768	0.0321	0.7075
Males	2.0067	0.049	0.1031	1.6542	0.8664	0.945	0.0134	0.8665

Implementation of any of the slopes of MR_L vs. stroking rate tested by the sensitivity analyses to the O_2 model with a set O_2 uptake curve resulted in a better correlation between estimated E_{O2} and stroking rate over 15 min intervals than using fixed E_{O2} per breath.

3.3.3.2. Basal Metabolic Rates

By applying a BMR, and so also an intercept, that was 2.7 times higher than the used BMR, the fit of the O_2 model including an uptake curve decreased by 15.4 % and 60.6 % for males and females, respectively (Table 3..8). Model fit of the female data was more sensitive for this considerable increase of BMR compared to the male data (Table 3.8). Model fit kept increasing and residual error decreasing with decreasing BMR for both sexes (Table 3.8). Other than the model fit of the female data in relation to the highest BMR, model slope stayed constant and was similar to the slope of MR_L vs. stroking rate for both sexes (Table 3.8, Fig. 3.11).

Increasing the BMR values implemented in the model with a set O_2 uptake curve for male 09_144b, would result in more breaths taken up maximum E_{O2} while the occurrence of breaths with lower E_{O2} would be more equal (Fig. 3.12). With decreasing BMR the frequency of breaths taking up maximally would decrease and a peak in occurrence would appear for E_{O2} between 5 and 8 L per breath (Fig. 3.12).

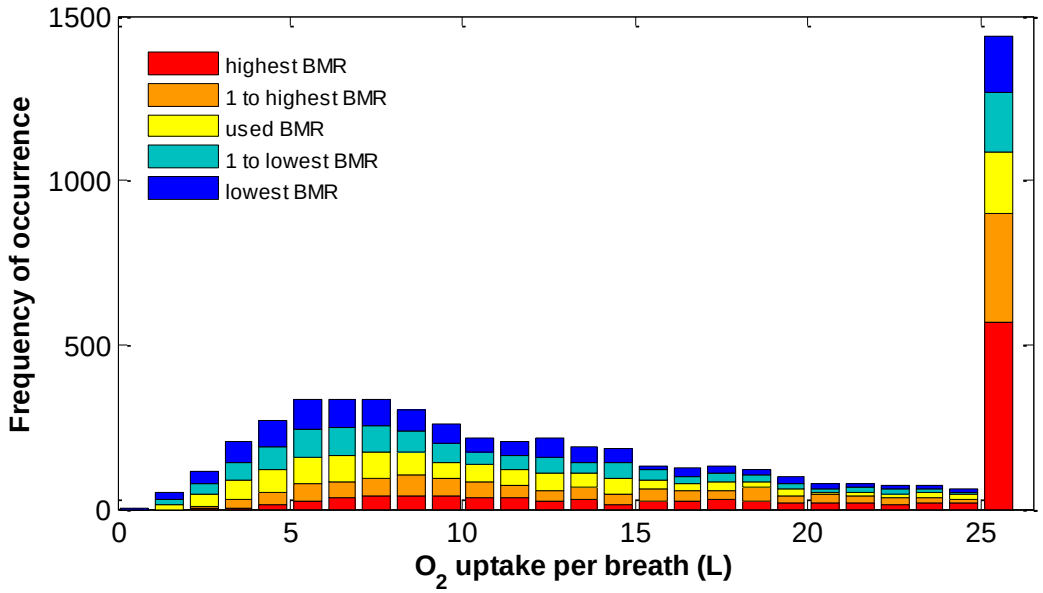

Figure 3.12. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for basal metabolic rate (BMR) incorporated into the O_2 model with a set O_2 uptake curve.

Also, the BMR was optimized according to the model with the fixed E_{O2}, as used by Kriete (1995) and Williams and Noren (2009), as explained in Section 2.3.4.2, and was on average 0.2346 and 0.4573 for females and males, being 3.2 and 4.4 times higher, respectively, than the used BMR. Applying these BMR values to the O_2 model with a set O_2 uptake curve did not produce a correlation between E_{O2} and stroking rate for females, but produced a moderate correlation for males (Table 3.10). BMR optimized according to the optimized fixed E_{O2} per breath was lower for both categories counting on average 0.1393 and 0.1316 for females and males, being 1.9 and 1.3 times larger, respectively, than the used values. Though by using these values slopes approached the slope of MR_L vs. stroking rate and model fit was high, the original BMR values still produced somewhat better model results (Table 3.10).

Table 3.10. Regression statistics for O_2 uptake estimated over the 15 min intervals with the O_2 model using a set O_2 uptake curve and optimized basal metabolic rate according to the O_2 model with fixed and optimized fixed O_2 uptake, versus level of activity represented by stroking rate over 15 min intervals per sex category. Intercept was set at same BMR as tested.

	Optimized BMR (fixed E_{O2})			**Optimized BMR (opt. fixed E_{O2})**		
	r^2	**RMSE**	**Slope**	r^2	**RMSE**	**Slope**
Females	0.092	0.0481	0.2130	0.879	0.0172	0.4686
Males	0.250	0.0614	0.5786	0.938	0.0111	0.6749

In summary, applying any of the BMR values tested by the sensitivity analyses to the O_2 model with O_2 uptake curve showed better model fit than the O_2 model with the optimized fixed E_{O2}.

3.3.3.3. Maximum O_2 Uptake per Breath

The correlation between estimated E_{O2} and stroking activity was high for any of the tested values for maximum E_{O2} (Table 3.8). A maximum E_{O2} of 2.1 and 2.3 times larger than the original values for males and females, elevated the model fit by 1.9 % and 1.2 %, respectively (Table 3.8). Whereas a maximum of E_{O2} of 25.3 % and 23.8 % lower than the original values for males and females, made the model fit decrease by 3.0 % and 13.2 %, respectively (Table 3.8). Slope stayed rather constant for both males and females (Table 3.8, Fig. 3.11). Residual variance increased slightly with decreasing maximal E_{O2} (Table 3.8, Fig. 3.11).

The higher the maximum possible E_{O2}, the less frequent a whale would take up maximally per breath (Fig. 3.13). The occurrence of all various E_{O2} amounts per breath under the lowest maximum E_{O2} value applied would be the same for applying any of the maximum E_{O2} values (Fig. 3.13).

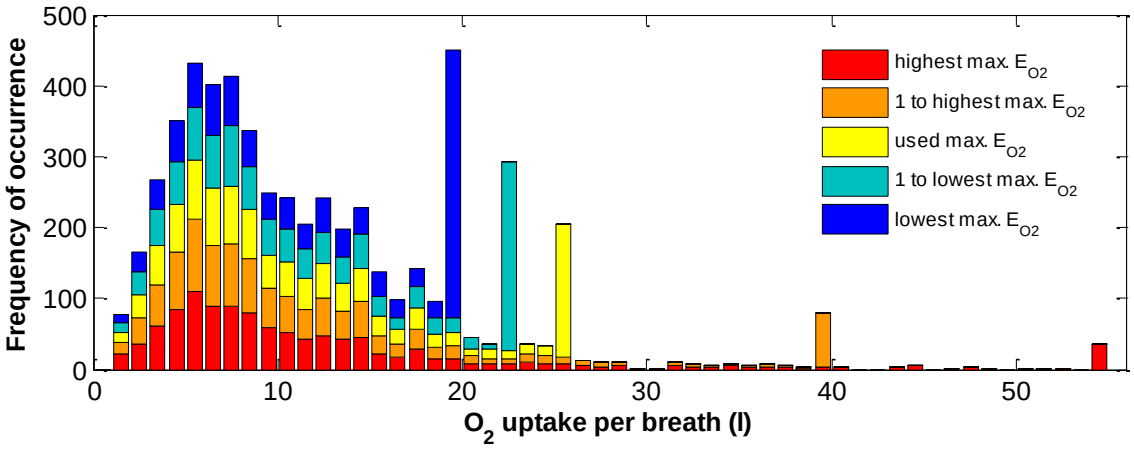

Figure 3.13. Occurrence distribution for the amount of E_{O2} per breath in male 09_144b according to different values for maximum possible O_2 uptake per breath incorporated into the O_2 model with a set O_2 uptake curve.

In summary, the sensitivity analyses results showed that the slope and the strength of the correlation between the V_{O2}, estimated with the O_2 model including a set O_2 uptake curve, versus stroking rate were especially sensitive to change of the slope of MR_L vs. stroking rate, in particular when these were higher than the values used. Female data was more sensitive to parameter value change. Though parameter values were uncertain in the O_2 model including the O_2 uptake curve these results supported the earlier conclusion that there is a better balance between estimated E_{O2} and V_{O2} over 15 min intervals when applying a set O_2 uptake curve to the model instead of assuming a fixed E_{O2} per breath, over a wide range of possible parameter values.

3.4. Discussion

3.4.1. Evaluation of the predictive power of the different O_2 models

As noticed in the previous chapter, the small-scale variation in activity was ignored due to application of 15 min intervals. Especially for stroking rates this is an accurate matter seen the swimming mode can change constantly and could include periods of gliding. Again, due to the typical diving behaviour of the whales, it would not be valuable to fit regressions between activity and E_{O2} for too short time intervals due to a delay in O_2 replenishment through breathing related to energetic demands by activity. Applying too short time intervals would result in a weaker or no correlation between the stroking and E_{O2} (Fig. 3.14).

3.4.1.1. The O_2 model including fixed and optimized fixed O_2 uptake per breath

Estimated MR for the 15 min interval from fixed and optimized fixed E_{O2} was relatively high and not in proportion to the BMR. Only for two males did there exist a moderate correlation between estimated MR and stroking rate over 15 min intervals using the fixed E_{O2} and setting the intercept at BMR, while for all other individuals and categories the estimated V_{O2} did not correlate with stroking rate at all. By not setting the intercept this correlation increased considerably for most individuals and for all females together. However, only for two females and two males the r^2 values were higher than 0.5. Moreover, one of these females exhibited a negative slope between estimated MR and stroking activity, plus intercepts for almost all individuals and both categories were unrealistically high compared to expected values for BMR (see Section 2.4.6.4 and 2.4.6.5). Similar results were found for using the optimized fixed E_{O2}, only MR estimates were somewhat lower, especially for the males, due to the lower fixed E_{O2} and therefore also slope coefficients were smaller. Intercepts were still considerably higher than BMR when not setting the intercept.

The O_2 store estimated according to the fixed E_{O2} kept increasing unrealistically high and unlimited, indicating that the E_{O2} was much higher than the V_{O2} per 15 min interval according to stroking activity and the slope of MR_L vs. stroking rate (Appendix 3.3). Optimized fixed E_{O2} was 1.3 and 1.9 times smaller for females and males, respectively, than the fixed E_{O2} applied by

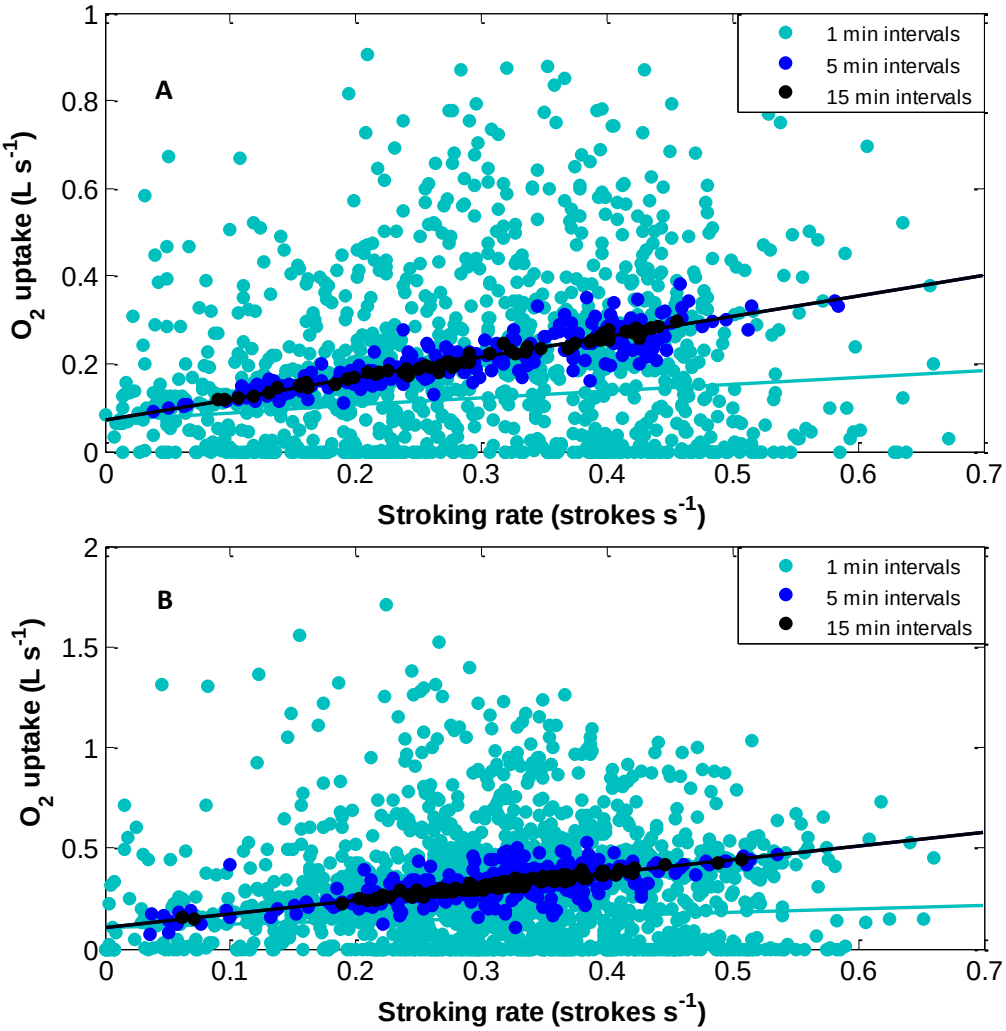

Figure 3.14. Oxygen uptake versus stroking rate, both averaged over 1, 5 and 15 min intervals, estimated through the model with fluctuating O_2 uptake according to a set O_2 uptake curve for female (A) and male (B) killer whales. Solid lines represent the O_2 model fitted with intercept set at BMR for the implementation of 1 min intervals (females: r^2 = -0.204, RMSE = 0.2078, coefficient = 0.1598, males: r^2 = -0.437, RMSE = 0.2889, coefficient = 0.1547), 5 min intervals (females: r^2 = 0.747, RMSE = 0.0307, coefficient = 0.4672, males: r^2 = 0.425, RMSE = 0.0564, coefficient = 0.6776), and 15 min intervals (females: r^2 = 0.979, RMSE = 0.0072, coefficient = 0.4690, males: r^2 = 0.950, RMSE = 0.0099, coefficient = 0.6749).

Kriete (1995) and Williams and Noren (2009). O_2 store estimated according to optimized fixed E_{O2} resulted as foreseen in a balance between E_{O2} and V_{O2} over the entire tag records, yet generally not during the 15 min intervals, and occasionally exceeded TBO capacity or became negative (Appendix 3.3). Optimized fixed E_{O2} for males was 50.4 % lower than the fixed E_{O2} in relation to mean V_T as measured by Kriete (1995). However, for females this optimized value was 30.6 % higher than fixed E_{O2} in relation to mean V_T measured by Kriete (1995).

In summary, it seemed that the fixed E_{O2} value was too high for both sexes, especially for

males. Moreover, as was shown for the model including speed as activity metric (see Chapter 2), multiplying respiration rate with this fixed uptake was not a good reflection of the true amount of O_2 used over 15 min intervals.

Weak statistical evidence existed that respiration rate was higher for males than for females for any stroking activity. To the contrast, Williams and Noren (2009) found weak statistical evidence that females had a higher respiration rate than males for all speeds explaining this finding by sexual dimorphism in killer whales, with females having higher mass-specific MR. However, the present study results revealed that respiration alone is not a reliable indicator of MR and therefore this interpretation is questionable. Moreover, evidence for both trends was weak, plus the present results showed a rather large overlap of respiration rates of both genders.

3.4.1.2. The O_2 model including a set O_2 uptake curve

Having the E_{O2} fluctuate in relation to the O_2 store at time of each breath made the correlation between estimated E_{O2} and V_{O2} over 15 min intervals increase considerably for both categories and all individuals. Overall MR estimated over 15 min intervals was substantially lower for both categories compared to applying a fixed E_{O2} value.

Given the difference in MR estimated through fixed E_{O2} and according to fluctuating E_{O2}, it appeared that average estimated E_{O2} over 15 min was significantly lower than the fixed E_{O2}, especially for males. Though the range of MR estimated through the model including a set O_2 uptake curve was comparable to MR estimated with optimized fixed E_{O2}, there was a tighter positive linear correlation between E_{O2} and V_{O2} over 15 min intervals for the former model variant, which was not present using the optimized fixed E_{O2}.

Thus, model results revealed that taking breath timing into account and having E_{O2} fluctuate according to O_2 store at time of breath, influenced MR estimates and so improved the correlation between E_{O2} and V_{O2} significantly. Therefore it can be concluded that allowing the E_{O2} to fluctuate per consecutive breath is essential to produce accurate MR estimates that correlate well with activity levels, though the precise value of MR remains somewhat uncertain due to parameter uncertainty.

3.4.1.3. The O_2 model including an optimized O_2 uptake curve

By optimizing the O_2 uptake curve applied to the model the correlation between estimated E_{O2} and V_{O2} over 15 min intervals improved for two of the six females and five of the six males. According to the optimized exponent values of the density dependent curve, it could be argued that according to the used maximum E_{O2}, TBO and the slope of MR_L vs. stroking rate the optimized uptake curve of the females together and individually is expected to resemble their non-optimized uptake curve. Whereas, the optimized curve for males together and individually appeared to be less similar to the non-optimized curve and decreased more gradually for high O_2 store values (Fig. 3.8). In contrast to the uptake curve optimization using speed as activity metric in the model, the optimization of sex categories was similar to the optimization results of the individual whales. Even though the density dependent O_2 model is simplistic and therefore optimization results should be handled with care, results showed that there potentially exists difference in the relationship between E_{O2} per breath and O_2 store not merely between genders, but also amongst individuals of the same gender. These differences could be caused by physiological, biological and ecological factors.

3.4.2. Stroking rate as an activity metric

The overall results of this study indicate that stroking rate derived from accelerometer data (as recorded by the DTAG) has potential to function as an activity metric in the O_2 model established in this study. However, when evaluating stroke amplitude and magnitude this method potentially would not suffice, as each stroke is weighted equally in terms of metabolic cost. The found average stroking rate for all killer whales together of 0.31 strokes·s^{-1} was somewhat lower than the mean stroke cycle frequency of 0.43 strokes·s^{-1} found by Sato *et al.* (2007), reflecting the presence of gliding periods. Though the difference found in average stroking rate between males and females was not significant, it was unexpected that stroking rates overall were higher for males. Stroke cycle frequency declines with body size across cetaceans (Sato *et al.*, 2007) so this seemed to indicate that males glide less, which is of interest to focus on in future research. Stroking measurements of all individual killer whales included multiple outliers or inaccuracies, of which some were extreme. Outliers were replaced with average stroke measurements, which potentially deviated from stroking activity at the time in the record before and after the outlier. For future studies it is proposed to

replace these outliers with average measurements of smaller intervals around the outlier. Nevertheless, for the correlation between estimated E_{O2} and stroking activity was evaluated over 15 min intervals, it was foreseen the method used to replace outliers did not influence the model results significantly.

The maximum measured stroking rate of 0.51 strokes·s^{-1} averaged over 15 min was lower than the implemented maximum in the "*findflukes*"-function of 2.5 strokes·s^{-1}. The minimum stroking rate measured of 0.06 strokes·s^{-1} average over 15 min was lower than the 0.2 strokes·s^{-1} implemented as the minimum rate in the "*findflukes*"-function. This latter observation could potentially be explained by the fact that all whales exhibited periods of non-stroking, so-called "gliding". These periods of zero stroking rate potentially has significant influence on the overall stroking rate per 15 min intervals, depending on how long and often the whale glided. Preferred swimming behaviour, including stroke cycle frequency, is unique per individual, depending on body size (Sato *et al.*, 2007) and speed is related to stroking rate (Fish, 1998) (see Section 4.2). Still, though minimum and maximal stroke duration was set for all individuals overall, nominal stroking rate and the minimal magnitude threshold were adapted per individual. Besides, body conditions affect buoyancy, and therefore also expected stroking activity (Sato *et al.*, 2003; Sato *et al.*, 2013).

During the present study it was assumed that every fluke stroke was of comparable strength and amplitude, and therefore comparably metabolic cost. Though, for some cetaceans it has been shown that stroke amplitude decreased linearly with swimming speed, it has been observed to remain constant with velocity for killer whales (Fish, 1998). Nevertheless, stroke magnitude was expected to influence O_2 use and should be considered in future research to improve upon the usage of stroking rate alone as an activity metric in the O_2 model.

3.4.3. Metabolic rates

As expected, the sensitivity results produced similar results in relation to maximum E_{O2} and BMR as for speed as an activity metric implemented in the O_2 model with O_2 uptake curve (see Section 2.4.6.3 and 2.4.6.4, respectively, for the discussion of these parameters).

No information existed on O_2 cost per stroke for killer whales at the time of the present study,

so used slope of MR_L vs. stroking rate could have been different from its true value. However, Williams *et al.* (2004a) produced important information on the energetic cost per stroke in a marine mammal, the Weddell seal: their estimation of cost per stroke was the single documented alternative value for slope of MR_L vs. stroking rate to implement within the O_2 model developed in this current study. When applying this energetic cost per stroke in the O_2 model with O_2 uptake curve, the correlation between estimated E_{O2} and stroking activity over 15 min intervals was somewhat lower for both sexes compared to applying the used values, yet still significantly strong. Still, the difference between used values and values extrapolated from findings by Williams *et al.* (2004a) was rather high, with the original values being 7.0 and 6.3 times as high for males and females, respectively. Because the O_2 store was depleted less swiftly when using the lower slopes of MR_L vs. stroking rate, E_{O2} per breath overall was lower with maximum E_{O2} per breath occurring less frequently than when applying the original value. Since it was expected that killer whales, as all other divers, deplete their O_2 store optimally to optimize E_{O2} per breath, the lower slopes of MR_L vs. stroking rate as extrapolated from Williams *et al.* (2004a) seemed less realistic for all the uptakes would be relatively low. However, this can be concluded merely in relation to the O_2 model developed with uptake curve and parameters values used. Whereas, when TBO capacity would be fixed at a lower value, the E_{O2} efficiency per breath would be higher using the slope of MR_L vs. stroking rate according to Williams *et al.* (2004a) and perhaps the used slope would be too high.

The true value of the slope of MR_L vs. stroking rate (*k*) remains undetermined. Nevertheless, it can be concluded that by either using the original slopes of MR_L vs. stroking rate, or the ones extrapolated from findings by Williams *et al.* (2004a), both resulted in a greater correlation between estimated E_{O2} and stroking activity over 15 min intervals, and had more balance estimated O_2 stores (Appendix 3.3), when applying a set O_2 uptake curve to the model to allow the uptake to fluctuate according to the O_2 store at the time of each breath, rather than assuming a fixed E_{O2} per breath. The stronger correlation is expected as the models including an uptake curve (either set or optimized) calculated V_{O2} based upon activity levels (stroking or speed). Ultimately, the value of the O_2 uptake models presented here can only be validated by related predicted metabolic rates to measured rates. It would be particularly useful to measure E_{O2} in blow expirate, and relate it to predicted values from the O_2 model by measuring whale activity levels using tags. Such validation should be feasible in some captive settings, but remain difficult to conceive for free-ranging animals.

4. General discussion

4.1. The significance of respiration timing in energetic estimates

Though studying energetics in marine mammals at sea is a challenge, it is important to make estimates of their individual and population energetic requirements to evaluate their top predator roles in food-webs and for establishing sustainable marine ecosystem management. During previous studies important gaps in knowledge required for estimating MR included V_T, E_{O2}, consecutive dives, fine-scale under water behavioural changes, and respiration timing. According to the results of the current study it can be concluded that allowing the E_{O2} to fluctuate per breath according to O_2 store, by accounting for breath timing and V_{O2}, is essential to produce MR estimates that correlate well with activity levels.

It can be anticipated that MR estimations derived during studies that used solely respiration rate might contain large inaccuracies caused by these flaws. Nonetheless, MR estimations derived according to respiration rate alone are used to estimate daily energetic expenditure of individuals or entire cetacean populations (Blix and Folkow, 1995; Kriete, 1995; Lafortuna *et al.*, 2003; Williams and Noren, 2009). Lafortuna *et al.* (2003) stated, according to MR estimations by respiration rate alone, that a foraging fin whale uses 1.13 $kJ \cdot kg^{-1} \cdot h^{-1}$ more compared to when not foraging. These authors also concluded that according to these energy requirements estimated through respiration rate this species was expected to consume 1.5 to 1.8 tons of krill on its summering ground to cover its daily budget (Lafortuna *et al.*, 2003). Blix and Folkow (1995) concluded that, according to the relatively low respiration rate compared to other species, the energy expenditure of minke whales is “low and fairly stable regardless of the (normal) behaviour of the animal”. However, seen the present study outcomes it could be interpreted that a relatively low respiration rate can reflect higher E_{O2} efficiency per breath, which would contradict the conclusion drawn by Blix and Folkow (1995). Blix and Folkow (1995) interpreted the “minor differences” in respiration rates of different minke whale populations in relation to pursued prey in different areas. Moreover, they used fixed E_{O2} and V_T from other species and argue that there exists only limited variation in these parameters. Besides, Blix and Folkow (1995) estimated the amount of prey needed to consume to

compensate for the energy expenditure estimated according to respiration rates alone. The present study is unique in the sense that it is based on fine-scale longitudinal data on simultaneous underwater movements and timing of breathing events of free-ranging killer whales collected through telemetry, while implying E_{O2} dynamics. In relation to the present study results it is recommended that existing MR estimations made according to merely respiration rate should be seriously questioned and handled with care during interpretation and drawing firm conclusions on food requirements and impact on ecosystems by killer whales and other cetacean species.

Increased time spent travelling/foraging was expected to increase the energy requirement for killer whales (Noren, 2011), especially for females (Williams and Noren, 2009). However, behavioural state within an individual defined not only MR, but also respiration timing. Every diving mammal has to make decisions concerning dive depth, dive duration, swim speeds, ascent/descent angles and foraging strategies while submerged (Thompson *et al.*, 1993). Diving animals could minimize surfacing time and maximize foraging efficiency by starting a dive with just sufficient O_2 for during the dive, so that they surface with an optimal depleted O_2 store which is refilled rapidly due to a high diffusion rate between the body and inhaled air (Kramer, 1988; Thompson *et al.*, 1993). Within behaviour classes it is expected to observe consistent diving patterns, depending upon the level of activity (Fedak and Thompson, 1993).

When comparing respiration behaviour of a high level activity period (porpoising) with a period of low level activity in male 09_144a, breaths were relatively equally distributed over time during the high-level activity period, breath intervals were longer on average, and the E_{O2} was greater (Fig. 4.1). This could be explained by the O_2 uptake curve in combination with behavioural state: while porpoising the animal is driven by the motivation to move fast (in this case as a response to sonar exposure; Miller *et al*., 2014) and therefore breathe in the most efficient way, while staying under the sea surface as much as possible to minimize surface drag costs. High average E_{O2} efficiency per breathe is reached through weighing the combination of apnea duration and MR. Killer whales display porpoising behaviour during fast travel, which will be costly, but still more efficient than sustaining high speed swimming just under the surface (Blake, 1983; Ford *et al.*, 2005). Au and Weihs (1980) revealed that when an animal exceeds a certain 'crossover' swim speed, and therefore high MR, leaping is more efficient than swimming continuously close to the surface in the need of air. However, during lower

activity periods E_{O2} was maximal for the first couple of breaths and decreased gradually according to replenishment of the store; E_{O2} during the end of the breathing bout was not efficient at all (Fig. 4.1). A possible purpose of these later breaths could be delayed CO_2 offload rather than O_2 uptake. In conclusion, it was shown that this male killer whale changed its breathing behaviour to influence E_{O2} per breath depending on its behavioural state in a manner that appears to be functional.

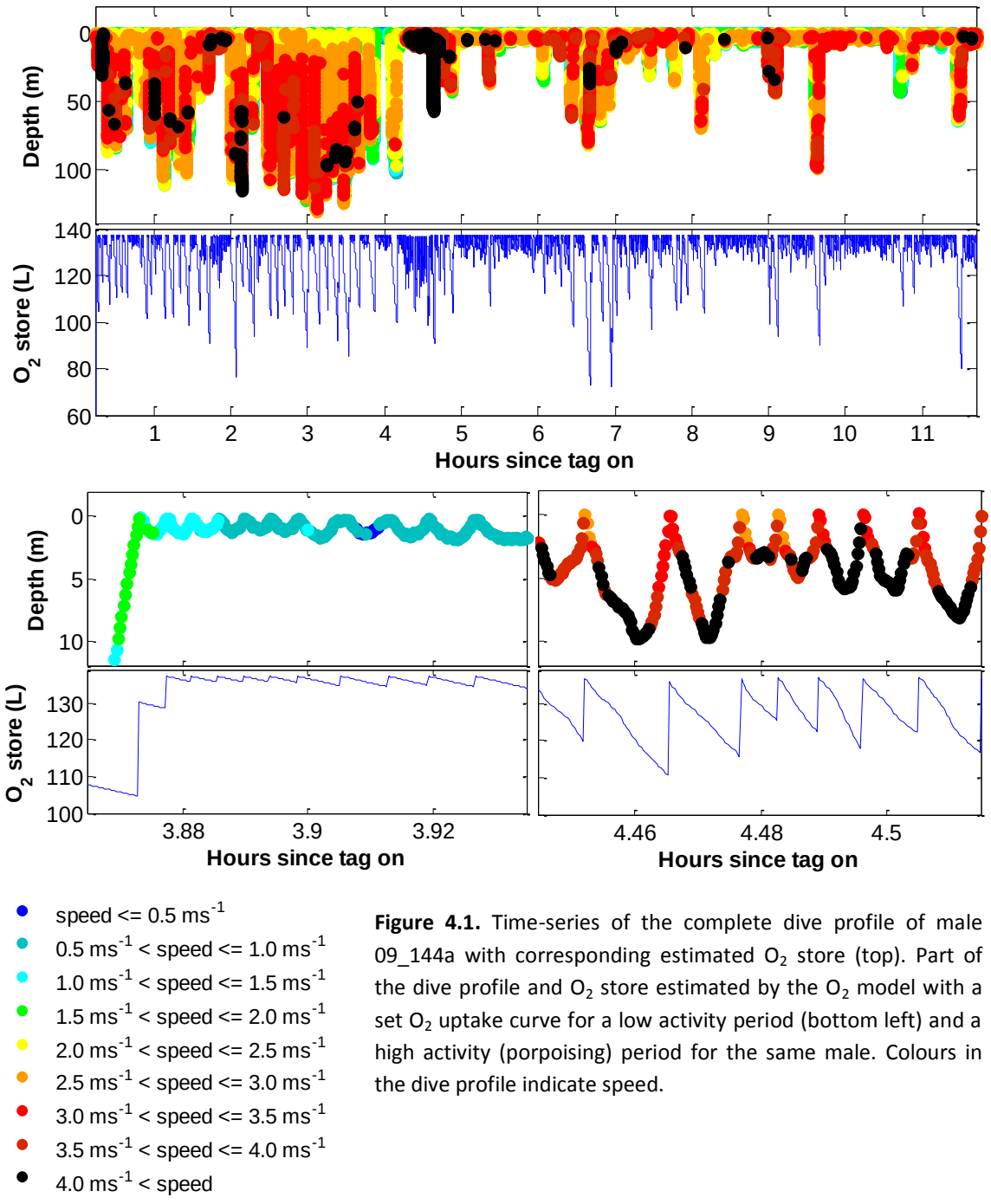

Figure 4.1. Time-series of the complete dive profile of male 09_144a with corresponding estimated O_2 store (top). Part of the dive profile and O_2 store estimated by the O_2 model with a set O_2 uptake curve for a low activity period (bottom left) and a high activity (porpoising) period for the same male. Colours in the dive profile indicate speed.

Also, COT has been derived from respiration rates in various studies on free-ranging cetaceans in which it was generally assumed that swimming effort is reflected in respiration rate (Sumich, 1983; Rodríguez de la Gala-Hernández *et al.*, 2008; Williams and Noren, 2009; Christiansen *et al.*, 2014). However, the present study results showed that COT and optimal swim speed estimations are less effective using respiration rates alone and ignoring timing of breaths and so fluctuation of E_{O2} per breath.

4.2. Using speed in contrast to stroking as activity metric

Both speed and stroking rate, both derived from DTAG data, showed potential to function as an activity metric in the developed O_2 model. The outcomes of the current study were that it was possible to derive realistic estimates of fluctuations in O_2 stores according to both activity metrics in combination with the physiological parameters used. Still, the slopes of MR_L vs. speed^3 (Chapter 2) or stroking rate (Chapter 3) have yet been determined during the present study and should be examined in future research.

4.2.1. Speed and stroking activity measurements

In relation to the available DTAG data it was chosen for this study to estimate V_{O2} through speed derived from flow noise and stroking rate. It was possible to extract other activity metrics from these data, including ODBA (Wilson *et al.*, 2006; Halsey *et al.*, 2009). However, its accuracy as an activity metric is questioned due to sensitivity to tag placement on the animal and movements of the tag during deployments (Noda *et al.*, 2012; Arnould *et al.*, 2014). In contrast to ODBA, stroking rates were not affected by tag placement, yet tag placement can have great impact on flow noise SPL of which speed is derived. Still, speed was retrieved uniquely per individual and periods between tag movements within individuals to account for differences in the relationship between speed and SPL due to tag placement. Speed showed a more distinguishable change over the tag records compared to stroking activity, exhibiting a wide distribution of speeds (Appendix 2.1 and 3.1). The killer whales' speed increased with stroking rate (Fig. 4.2). However, when an animal stops stroking it can still show forward movement during gliding, and so stroking rate can potentially vary greatly on a fine time-scale whereas speed fluctuates more smoothly. Outliers in stroking measurements were

considerably more extreme than the outliers for speed (Appendix 2.1 and 3.1). As body size and condition affect buoyancy every single cetacean exhibits a unique swimming behaviour and preferred stroking rate (Sato *et al.*, 2007). Though stroke rate measurements are robust to tag placement by setting individual thresholds, measurement of the amplitude of strokes is affected by tag placement and is less easily accounted for using current methods.

4.2.2. Knowledge on metabolic rates in relation to speed and stroking

At the time of current study there was more data available for cetaceans on how swimming speed relates to energetics requirements compared to how stroking activity relates to energetic requirements. Care should be taken when comparing speed estimated through animal surfacing locations with speed estimated from tag data. Whereas for speed there existed established formulae to estimate energetic cost according to speed (Fish, 1998; Guinet *et al.*, 2007) this was not the case for stroke rate. Only one study estimated metabolic cost per stroke in a pinniped species (Williams *et al.*, 2004a) and so the slope of MR_L vs. stroking rate used in the current study was less certain than the slope of MR_L vs. speed^3.

As stressed before by Williams *et al.* (1999) swim speed maintained by either continuous stroking or gliding can have different impacts on the O_2 store. However, though obviously gliding events were accounted for by estimating MR in relation to stroking activity, it was not accounted for by estimating MR according to speed. Therefore it was assumed that by applying this latter activity metric MR would be overestimated during gliding events.

4.2.3. Model outcomes according to stroking versus speed

By applying either one of the activity metrics (speed or stroking rate) to the O_2 model, it was concluded that respiration timing is essential to include in the model to vary E_{O2} per breath depending on the O_2 store at time of each breath.

For all females together the r^2 value of the correlation between estimated V_{O2} and E_{O2} per 15 min intervals was 0.08 higher when using stroking as activity metric included in the O_2 model with an uptake curve compared to when using speed (Appendix 4.1). For all males together

this correlation strength was more comparable using either stroking or speed (Appendix 4.1). In relation to this same model variant four of the females showed a somewhat tighter correlation between estimated E_{O2} and V_{O2} using speed, with an average difference in r^2 value of 0.021. One other female showed an increase of 0.023 of r^2 value by applying stroking (Appendix 4.1). Four males showed a tighter correlation when using stroking with an average increase of the r^2 value by 0.075 (Appendix 4.1).

In contrast, the optimized fixed E_{O2} uptake according to speed as activity metric (4.56 and 6.09 for females and males, respectively) was almost half of the one optimized according to stroking for both genders (8.93 and 11.9 for females and males, respectively). This result could either be explained by that the slopes of MR_L vs. activity (k), which potentially deviated from true values for both activity metrics, were not in relation to one another; or stroking was not directly related to speed. As discussed before, the true V_{O2} related to stroking and speed remains undetermined so no further conclusions could be drawn according to this aspect.

Though speed and stroking on a fine time-scale potentially can be poorly correlated, over 15 min intervals they were expected to be correlated according to level of activity overall. The r^2 values of these correlations counted 0.552 and 0.754 for males and females, respectively (Fig. 4.2). Though females have smaller flukes and therefore need to stroke more frequently to reach and/or maintain a certain speed compared to males (Sato *et al.*, 2007), it was expected that females would have a higher intercept and a similar correlation slope to the ones for males, rather than a higher correlation slope as it should be apparent for all speeds. While the intercept of this correlation for females was close to the origin, the intercept for males was not (Fig. 4.2). However, there existed rather large residual errors within gender categories caused by individuals, for the range of correlation slopes was relatively high for both genders (Table 4.1, Appendix 4.2). Some individuals with small sample size and exhibiting only a small range of speed resulted in suspicious slope coefficients and intercepts, whereas individuals which exhibited speed ranges of at least 2 $m \cdot s^{-1}$ and a minimum of ten samples revealed more realistic regression statistics (Table 4.1, Appendix 4.2). Of these latter individuals the highest coefficient was 0.150 exhibited by female 05_322a, while the coefficient for female 06_327s was 0.089 (Table 4.1, Appendix 4.2). The correlation slopes and intercepts found for males 09_144a and 09_144b were more similar to one another (Table 4.1, Appendix 4.2).

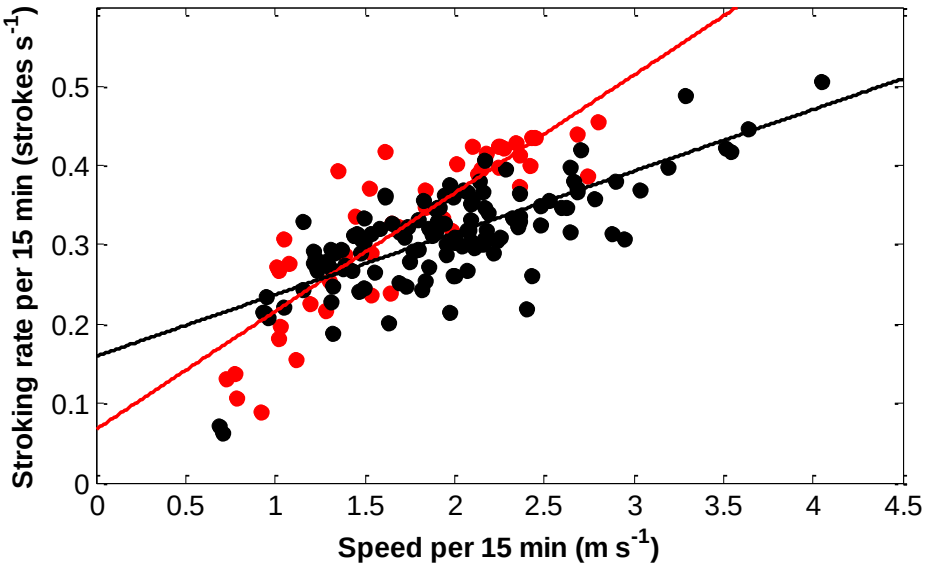

Figure 4.2. Correlation between stroking rate and speed per 15 min intervals for males (black) and females (red), with solid lines representing simple linear regression models with r^2 values of 0.552 (0.0778 * flukes + 0.1593) and 0.754 (0.149 * flukes + 0.0669) for males and females, respectively.

Table 4.1. Simple linear regression statistics and relationships between speed and stroking rate over the 15 min intervals per individual killer whale. Killer whales with a sample size of minimal ten and a minimum speed range of 2 $m \cdot s^{-1}$ are coloured.

	Gender	# 15 min blocks	r^2	slope	intercept	RMSE
05_316a	Female	7	0.976	0.190	-0.061	0.019
05_320b	Male	5	0.993	0.141	0.078	0.005
05_321b	Female	4	0.897	0.277	-0.091	0.017
05_322a	Female	11	0.714	0.150	0.085	0.062
05_322b	Male	9	0.659	0.195	-0.004	0.068
06_313s	Female	6	0.902	0.263	-0.125	0.023
06_327s	Female	19	0.813	0.089	0.206	0.024
06_327t	Male	20	0.002	0.005	0.265	0.036
09_144a	Male	44	0.679	0.079	0.182	0.026
09_144b	Male	47	0.621	0.085	0.123	0.037

As there existed a correlation between stroking and speed it was foreseen that overall model outcomes would be similar, yet actual continuous O_2 store estimates, and so estimated E_{O2} per 15 min intervals, could vary depending on the slope of MR_L vs. activity (k) determining V_{O2}. Also, continuous estimated V_{O2} per second differed according to activity metric due to the implemented energetic equations. It appeared that towards extreme activity levels the V_{O2} estimated through both metrics diverged; for high activity levels estimated V_{O2} was higher using speed, while for low activity periods stroking resulted in a higher V_{O2} estimate.

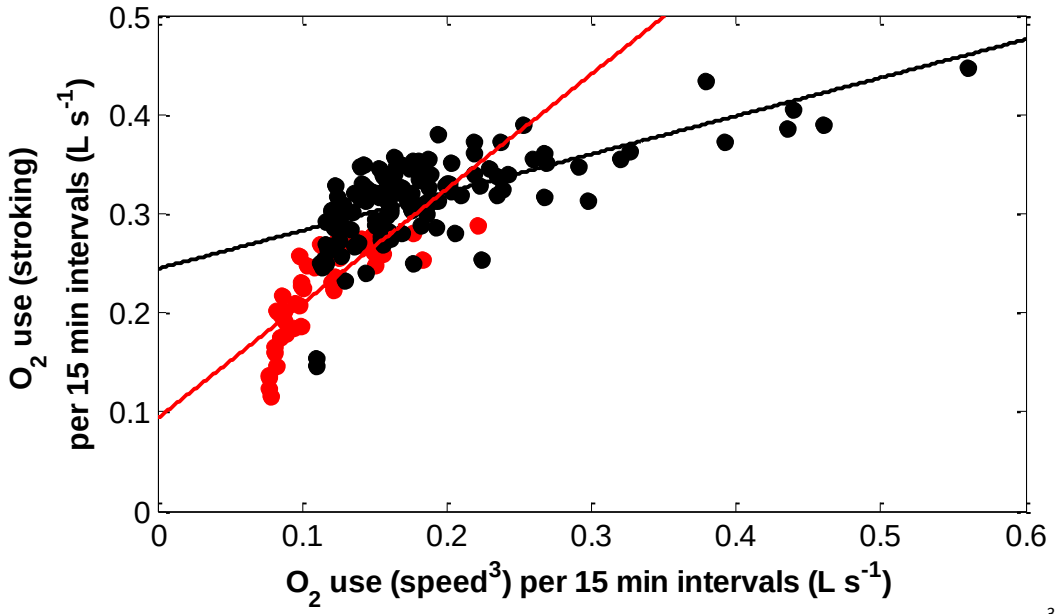

Figure 4.3. Correlation between O_2 use (including BMR) estimated according to stroking and speed^3 per 15 min intervals for males (black) and females (red), with solid lines representing simple linear regression models with r^2 values of 0.451 and 0.623 for males and females, respectively.

Table 4.2. Simple linear regression statistics and relationships between O_2 use estimated according to stroking activity and speed over the 15 min intervals per individual killer whale. Killer whales with a sample size of minimal ten and a minimum V_{O2} range of 0.1 L ·s^{-1} according to speed are coloured.

	Gender	# 15 min blocks	r^2	slope	intercept	RMSE
05_316a	Female	7	0.922	1.878	-0.014	0.017
05_320b	Male	5	0.934	1.435	0.086	0.010
05_321b	Female	4	0.920	5.179	-0.254	0.007
05_322a	Female	11	0.537	0.889	0.119	0.037
05_322b	Male	9	0.607	3.228	-0.151	0.049
06_313s	Female	6	0.908	2.943	-0.075	0.011
06_327s	Female	19	0.616	0.703	0.165	0.016
06_327t	Male	20	0.040	-0.266	0.324	0.024
09_144a	Male	44	0.605	0.413	0.260	0.020
09_144b	Male	47	0.633	0.369	0.239	0.025

V_{O2} estimated over 15 min intervals according to stroking ($V_{O2\ strokes}$) was expected to be equivalent to V_{O2} estimated over 15 min intervals through speed ($V_{O2\ speed}$), which would be reflected by a correlation with coefficient of 1.0 and intercept at the origin. However, V_{O2} estimated according to stroking and speed^3 was not equal and there existed a difference in correlation between genders when pooling individuals within gender (Fig. 4.3). Correlation strength between $V_{O2\ strokes}$ and $V_{O2\ speed}$ was 0.451 and 0.623 for males and females,

respectively (Fig. 4.3). According to the correlation found for females $V_{O2\ strokes}$ estimates per 15 min intervals were always higher than $V_{O2\ speed}$ estimates (Fig. 4.3). For males V_{O2} up to approximately 0.4 $L \cdot s^{-1}$, corresponding to a stroking rate of 0.4 $strokes \cdot s^{-1}$ and speed of 3.7 $m \cdot s^{-1}$, was higher estimated by stroking while for higher activity levels V_{O2} was estimated higher through speed (Fig. 4.3). The variance within categories could be explained by the rather large differences in correlation coefficient among individuals (Table 4.2, Appendix 4.3).

Only male 06_327t did not show a correlation between V_{O2} estimated from stroking rate and speed over 15 min intervals, whereas all other individual killer whales showed in general moderate to strong correlations (Table 4.2, Appendix 4.3). Individual whales with a sample size larger than 10 and a minimum V_{O2} range of 0.1 $L \cdot s^{-1}$ according to speed (including females 05_322a and 06_327s, and males 09_144a and 09_144b) exhibited slope coefficients ranging from 0.369 to 0.889 (Table 4.2). Slopes of both females were higher than of the two males, whereas intercepts of both males were higher than for females, as was similarly showed for categories (Table 4.2, Appendix 4.3). The range of activity level estimated for the 15 min intervals for the two females were lower than for the two males which could have significantly influenced slope coefficients (Appendix 4.3). These results and the fact that the intercept for both categories lies above the origin could also be the result of deviations in the slopes of MR_L vs. activity and/or specified BMR values. Regarding females, the sensitivity analyses revealed that correlation between estimated E_{O2} and activity level over 15 min intervals increased for lower slopes of MR_L vs. activity in particular for $speed^3$, whereas changing the slope of MR_L vs. activity for males would either have no effect or decrease the correlation strength for speed and stroking, respectively. Obviously, the correlation between the V_{O2} of the two slopes of MR_L vs. activity could be influenced by both and in combination with BMR values. Moreover, V_{O2} according to both metrics can differ per individual partially due to the unique correlation between stroking rate and speed per individual whale caused by differences in fluke size. For these reasons it was not pursued to draw conclusions regarding potential adjustment of these parameter values.

Nevertheless, although there remained uncertainty about absolute energetic values related to both activity metrics, this study showed that both activity metrics showed potential to be used to estimate kinematic costs revealing strong relationships between activity level and estimated E_{O2} over 15 min intervals for all individuals according to the established O_2 model in

combination with the used data on free-ranging killer whales. However, forward speed is produced by stroking so it is considered an indirect measure of kinematic costs whereas stroking is a body movement and therefore a more direct measure. Still, very few information existed on V_{O2} in relation to stroking at the time of the present study.

4.3. Example application of the O_2 model to test for energetic consequences by sonar

4.3.1. Introduction

Though killer whales have no natural predators, humans have a potential large impact on their behaviour and well-being through disturbance. Killer whales, like other cetaceans, rely on their vocalizations in order to survive and evolved a sensitive hearing over a wide range of frequencies, which in combination with the propagation of sound underwater would make them sensitive over large distances to anthropogenic noise (Würsig and Richardson, 2009). Chronic short-term disturbance can affect a population on the long-term, concerning habitat displacement, fitness of individuals and therefore reduced reproduction effecting the population as a whole (Morton and Symonds, 2002; Williams *et al.*, 2006).

Disturbance by boat presence can alter cetaceans' behavioural budgets and has a potentially negative impact on the energy intake of the animals (Williams *et al.*, 2002b; 2002a; 2006; Lusseau *et al.*, 2009; Williams *et al.*, 2009b). It has among other things been shown to affect swimming speeds, potentially increasing energy expenditure (Nowacek *et al.*, 2001; Williams *et al.*, 2002b). Killer whales shown more surface active behaviour during boat presence which potentially also can increase energy expenditure (Noren *et al.*, 2009). However, Williams *et al.* (2006), who applied energetic requirements per activity measured by Kriete (1995), concluded that although boat presence can affect the behavioural budget of killer whales, it is not the direct energetic costs related to these behavioural changes, but rather the decreased time spent on feeding which potentially implies a more significant burden concerning energetic demands. Also, it was hypothesized that it is not the boat presence itself, but rather the boat noise that causes disturbance (Williams *et al.*, 2006).

Though direct observations of sonar impact on cetaceans are rare (Kuningas *et al.*, 2013), it is feared that sonar has strong negative effects on cetaceans' physiology (physiological stress), including short-term emotional stress increasing standard metabolic rate and physiological burden caused by behavioural responses (behavioural stress) (Gartner *et al.*, 1980; Hildesheimer *et al.*, 1985; Chrousos, 2009; Miller *et al.*, 2014). Killer whales and other cetacean species exposed to experimental sonar tried to evade the source by increasing speed and changing direction (Miller *et al.*, 2011; 2014). Also foraging behaviour could potentially be interrupted and groups could be separated by the disturbance, including dependent calves (Lusseau *et al.*, 2009; Miller *et al.*, 2014). Other cetacean species exposed to experimental sonar tried to evade the source by increasing speed and changing direction (DeRuiter *et al.*, 2013).

Breathing rates (multiplied by a fixed E_{O2}) have been used as a metric to assess actual energetic expenditure, potentially caused by stress in cetaceans, ignoring breath-by-breath variation in gas exchange (Todd *et al.*, 1996; Kastelein *et al.*, 2001; Noren, 2006; Christiansen *et al.*, 2014). Noren (2006) found no significant correlation between breathing rate and boat presence concerning killer whales. Conversely, an elevated respiration rate in relation to anthropogenic disturbance was found for harbour porpoises (Kastelein *et al.*, 2001) and minke whales (Christiansen *et al.*, 2014). Christiansen *et al*. (2014) found that the increases respiration rate in minke whales, caused by presence of whale-watching boats, resulted in an increase of estimated energy expenditure by 27.6 %. Though they considered the simultaneous increase in swim speed as well, they concluded that this elevated activity level accounted for merely 15.9 % of the total increase of V_{O2} triggered by the presence of whale-watching boats (Christiansen *et al.*, 2014). Still, they acknowledge that the increase in respiration rate potentially reflected a change in V_T and E_{O2} efficiency, rather than an increase in metabolic rate (Christiansen *et al.*, 2014). In light of the study results presented in Chapter 2 and 3 of this thesis the accuracy of the findings of these studies, assessing energetic consequences by stress using respiration rate alone, is uncertain.

Still, measuring energetic costs of human stressors that cause behavioural disturbance, such as sonar, is important to evaluate its significance in both short- and long-term effects on individual survival. This knowledge is needed to help nations improve making their trade-offs regarding naval sonar exercise versus cetacean conservation. During this section of the thesis

short-term energetic consequences of behavioural responses to sonar in free-ranging killer whales were assessed using the DTAG data and applying the O_2 model including a set O_2 uptake curve as presented in Chapter 2 and 3.

Short-term physiological stress is expected to increase metabolic rate (Gartner *et al.*, 1980; Chrousos, 2009), so divergences in the E_{O2}-activity level correlations provide a tool to evaluate whether physiological stress might be occurring during sonar exposures. Such divergences of high estimated E_{O2} would indicate that a whale would use more O_2 than required for the same activity level under natural circumstances. Results were subsequently briefly evaluated and discussed in relation to outcomes of former studies on disturbance effects on metabolic rates in cetaceans using breathing rates alone.

4.3.2. Material and methods

Four of the tagged Norwegian herring-feeding killer whales included in this study were experimentally exposed to sonar during tag deployment. Before these individuals were exposed to sonar baseline data was collected to create a natural condition as control group (Miller *et al.*, 2011). For detailed information on the sonar experiments see Miller *et al.* (2011).

E_{O2} over 15 min intervals were estimated for the four individual killer whales according to both stroking and speed, using the established O_2 model using fixed E_{O2}, as used by Kriete (1995) and Williams and Noren (2009), and including a set O_2 uptake curve to correct E_{O2} per breath for TOB saturation at time of each breath.

4.3.3. Results

For female 06_327s there was no clear indication of behavioural disturbance during sonar; however her activity level post sonar was elevated (Fig. 4.4 and Fig. 4.5). Male 06_327t exhibited an above-average level of activity during sonar (Fig. 4.4 and Fig. 4.5). However, due to research logistics there were no 15 min interval measurements for this individual as control group. The two 15 min intervals for which the highest level of activity was measured for male 09_144a occurred during sonar exposures, while other measurements did not show deviation

from level of activity pre or post sonar (Fig. 4.4 and Fig. 4.5). For male 09_144b the highest stroking rate and speed was observed during sonar exposure, while all other activity level measurements did not show deviation compared to activity level pre and post sonar (Fig. 4.4 and Fig. 4.5).

Respiration rates calculated per 15 min interval during sonar exposures were not significantly higher than respiration rates during 15 min periods without sonar for any of the individual killer whales, irrespective of activity level (Fig. 4.4). Moreover, respiration rates during sonar exposure did not diverge significantly more from the fitted E_{O2}-activity correlations than respiration rates pre and post sonar for any of the individuals (Fig. 4.5).

The E_{O2} estimates per 15 min interval, corrected for TBO saturation at time of each breath by including a set O_2 uptake curve in the O_2 model, revealed that none of the individuals experienced elevated energetic costs during sonar exposures beyond those caused by potentially elevated activity levels: all E_{O2} estimates for 15 min interval with sonar exposure showed only small deviation from the E_{O2}-activity correlations for all individuals (Fig. 4.5).

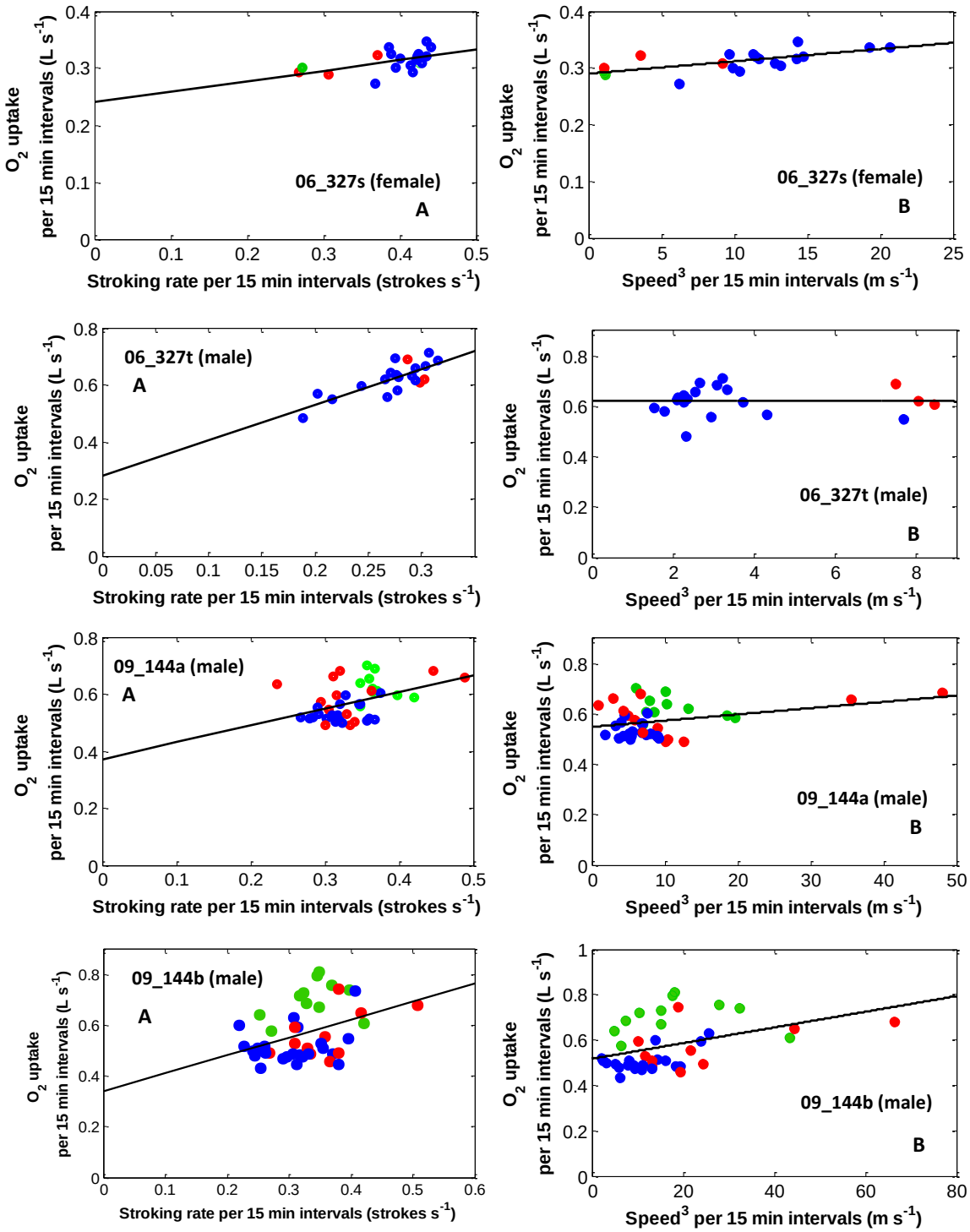

Figure 4.4. O_2 uptake per 15 min intervals versus stroking rate (A) and speed^3 (B) estimated through the O_2 model with fixed O_2 uptake for the individual adult killer whales which were experimentally exposed to sonar, including 15 intervals before exposure (green), during exposure (red) and post exposure (blue).

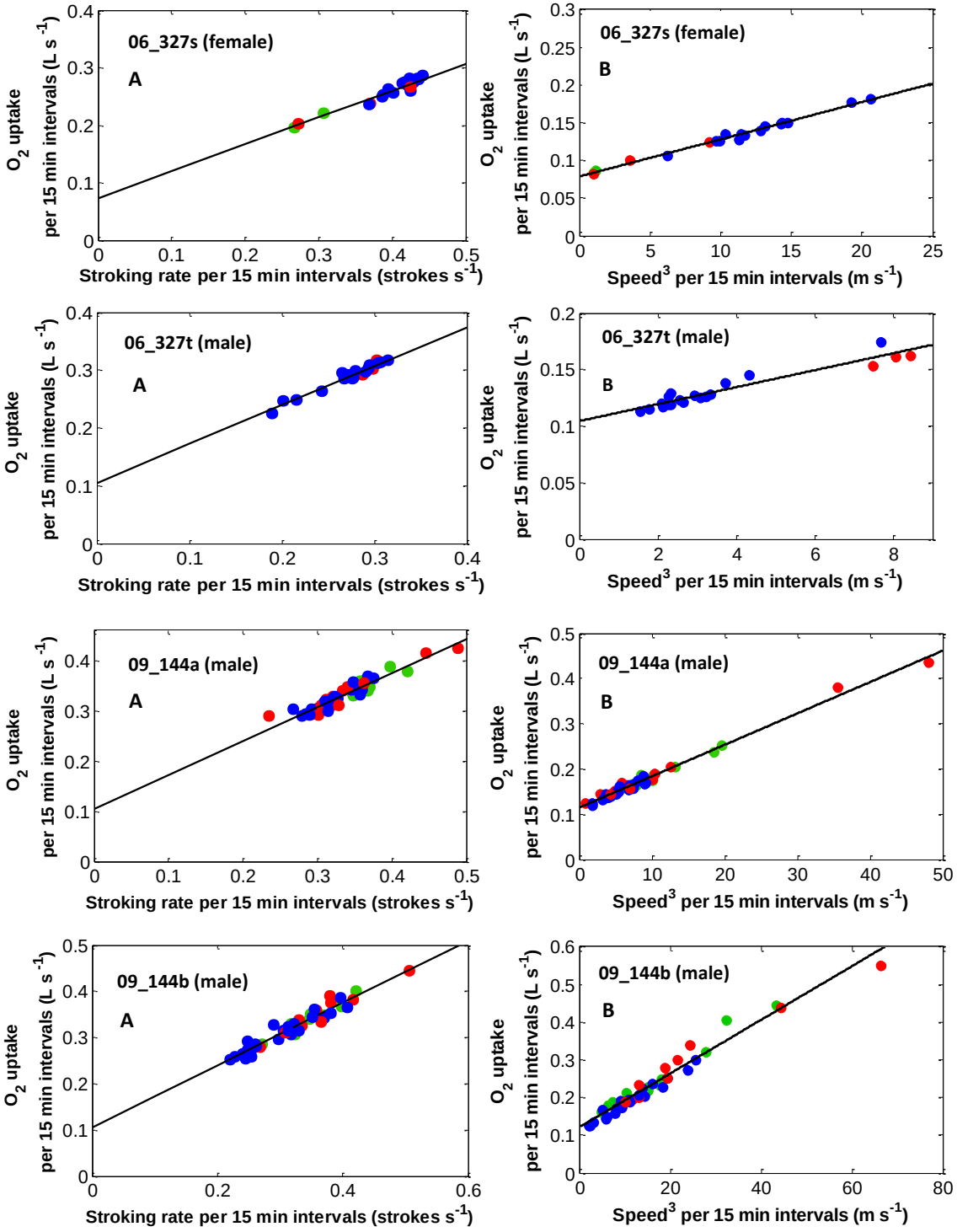

Figure 4.5. O_2 uptake per 15 min intervals versus stroking rate (A) and speed^3 (B) estimated through the O_2 model with a set O_2 uptake curve for the individual adult killer whales which were experimentally exposed to sonar, showing 15 intervals before exposure (green), during exposure (red) and post exposure (blue).

4.3.4. Discussion

According to these results it can be concluded that the potential response to sonar by killer whales is reflected rather by level of activity (behavioural stress) than elevated E_{O2} (physiological stress). Moreover, there existed individual differences in response to disturbance by sonar.

These results, in relation to using respiration rate, showed a similar outcome as found by Noren (2006); there was not an obvious signal of higher breathing rates during sonar exposures. However, applying the O_2 model with an uptake curve indicated that certain 15 min periods with high activity were also associated with elevated E_{O2}. This was particularly the case for high-speed travel periods when the tagged whales strongly changed their breathing patterns (see Fig. 4.1 for example). However, these elevated estimates of metabolic rate fell along the same trend-line as other 15 min periods, including periods without sonar, indicating there was not a large additional metabolic burden added by physiological stress when animals did respond to sonar. This observation accentuates the importance of the specification of V_{O2} in the model as key constraining factor. The lack of physical stress during boat presence in killer whales was previously revealed by Ayres *et al.* (2012) who measured glucocorticoids in faeces. However, regarding the potential behavioural stress sonar potentially causes a substantial decrease in energetic intake due to disruption of feeding behaviour, as shown for disturbance by vessel traffic (Lusseau *et al.*, 2009).

Still, the present analyses included only four individuals and more research is needed to draw significant conclusions in relation to energetic consequences due to disturbance in killer whales, for both boat presence and sonar. However, this method of estimating V_{O2} may have promise to provide more realistic estimates of energetic expenditure during disturbance, and could be applied to other cetacean species as well.

4.4. Conclusions

This study showed that an O_2 model including an O_2 uptake curve is a significant improvement over using fixed E_{O2} per breath (breathing rate) to derive realistic metabolic rate estimates from longitudinal observations of breath times and under water activity level on free-ranging killer whales. However, one important constraint of the presented model is the paucity of concrete measurements on V_{O2} in relation to both speed of travel and stroking rate in cetaceans. Therefore there would be a clear scientific benefit to collect such information directly from captive specimens as discussed previously in Chapters 2 and 3. It is necessary to validate, improve and extend this model to make it applicable to all cetacean species, and increased knowledge of the key gas-exchange parameters in other cetacean species would be helpful.

Findings of this study indicated that taking into account respiration timing, in addition to rate, is crucial in making accurate energetic estimations for free-ranging cetaceans. With on-going development and effort concerning bio-logging and its technology, in combination with gain of accurate information on energetic costs in relation to kinematics, the proposed model could become a useful tool to improve our knowledge of free-ranging cetaceans' energetic requirements and respiration behaviour in relation to more advanced sustainable marine ecosystem management.

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Appendices

Appendix 1.1. Latin names of species mentioned in text

Australian sea lion	*Neophoca cinerea*
Beluga whale	*Delphinapterus leucas*
Bottlenose dolphin	*Tursiops truncatu*s
California sea lion	*Zalophus californianus*
Emperor penguin	*Aptenodytes forsteri*
Fin whale	*Balaenoptera physalus*
Grey seal	*Halichoerus grypus*
Grey whale	*Eschrichtius robustus*
Harbour porpoise	*Phocoena phocoena*
Herring	*Clupea harengus*
Humpback whale	*Megaptera novaeangliae*
Killer whale	*Orcinus orca*
Minke whale	*Balaenoptera acutorostrata*
Northern fur seal	*Callorhinus ursinus*
Pacific white-sided dolphins	*Lagenorhynchus obliquidens*
Sea otter	*Enhydra lutris*
Southern sea lion	*Otaria flavescens*
Steller sea lion	*Eumetopias jubatus*
Walrus	*Odobenus rosmarus*
Weddell seal	*Leptonychotes weddellii*

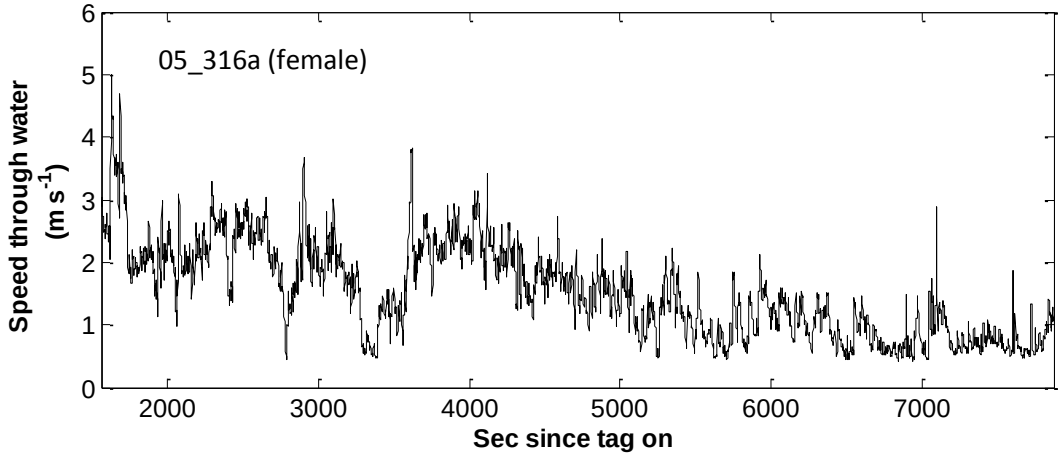
Appendix 1.2. Key studies conducted on large *in-situ* cetaceans to estimate metabolic rates via respiration rates and the integration of data collected in the captive environment. (BR = breathing rate)

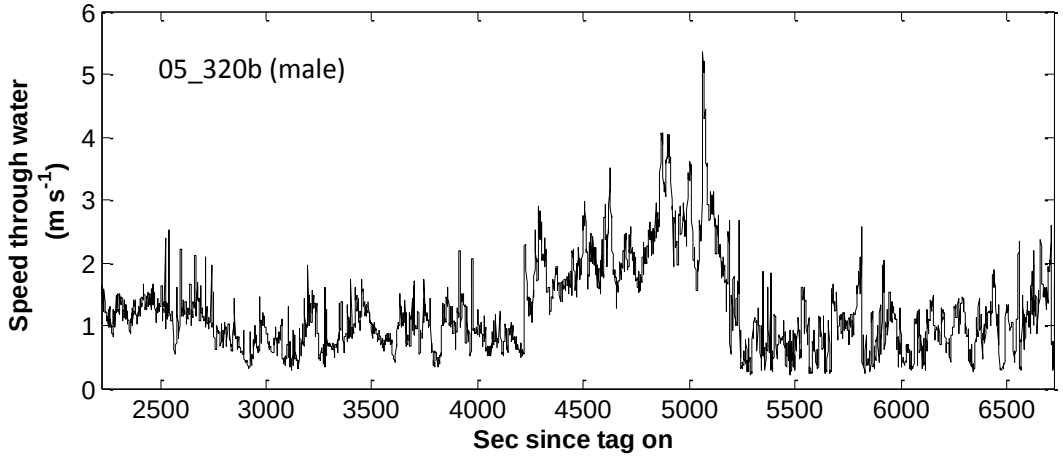
Author(s)	Study species	Research goal	Methods	Assumptions	Outcomes
Sumich (1983)	Grey whale	Discover possible relationship between BR and speed Estimate minimal COT	Measured speed and BR through 'surveyor's transit'	Surface positions refer to actual distance travelled Constant V_T Constant E_{O2} Developed blood capacities in immature individual	In particular apneas of <1min or >2 min Curvilinear correlation between BR and speed Min COT at 2.0-2.2 ms^{-1}
Dolphin (1987)	Humpback whale	Estimate V_{O2} related to diving behaviour	Counting BR during foraging behaviour	Constant V_T Constant E_{O2}	V_{O2} positively correlated with dive depth Minimal V_{O2} found for dive at 41-60 m in depth
Blix and Folkow (1995)	Minke whale	Estimate COT for different speeds and activities	Animals tagged and BR recorded	Constant V_T Constant E_{O2}	Curvilinear correlation between BR and speed MR independent of behaviour
Kriete (1995)	Killer whale	Discover relationship between BR and speed and between MR and speed Estimate COT for different speeds	Measured speed and BR through 'surveyor's transit'	One dive model to estimate distances for all tracks Constant E_{O2} per speed category	Linear correlation between BR and speed Curvilinear correlation between MR and speed Decreasing COT with speed
Rodríguez de la Gala-Hernández *et al.* (2008)	Grey whale	• Compare swimming effort between age class and productive status • Estimate calf COT	Measured speed and BR through 'surveyor's transit'	• Surface positions refer to actual distance travelled • Constant V_T • Constant E_{O2}	• Swimming effort was highest for calves and mothers • Calf COT ranged from 0.25-0.58 $J \cdot kg^{-1} \cdot m^{-1}$

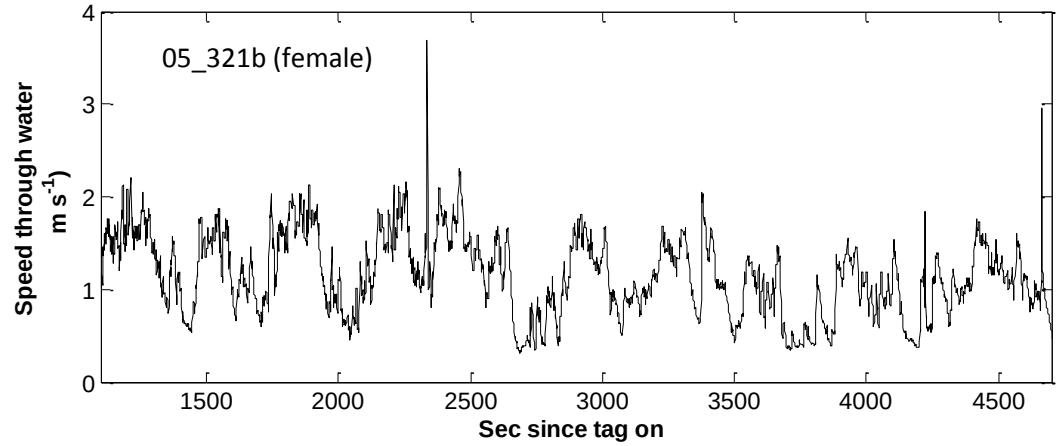
Appendix 1.2. Continued

Author(s)	Study species	Research goal	Methods	Assumptions	Outcomes
Williams and Noren (2009)	Killer whale	Estimate COT for different speeds	Measured speed and BR through 'surveyor's transit'	Surface positions refer to actual distance travelled to a certain extend Constant V_T Constant E_{O2} per activity level	Linear correlation between BR and speed Decreasing COT with speed
Christiansen *et al.* (2014)	Minke whale	Estimate COT for different speeds Discover relationship between BR and speed, and influence by whale-watching	Measured speed and BR through 'surveyor's transit' of non-feeding tracks	Surface positions refer to actual distance travelled Constant V_T Constant E_{O2}	Linear correlation between BR and speed, however swim speed explains only 6.5% of variance in BR Decreasing COT with speed as power function

Appendix 2.1. Time series plots of the speed measurements per individual killer whale before (A) and after (B) removing outliers. Individuals showing merely one plot did not exhibit outliers.







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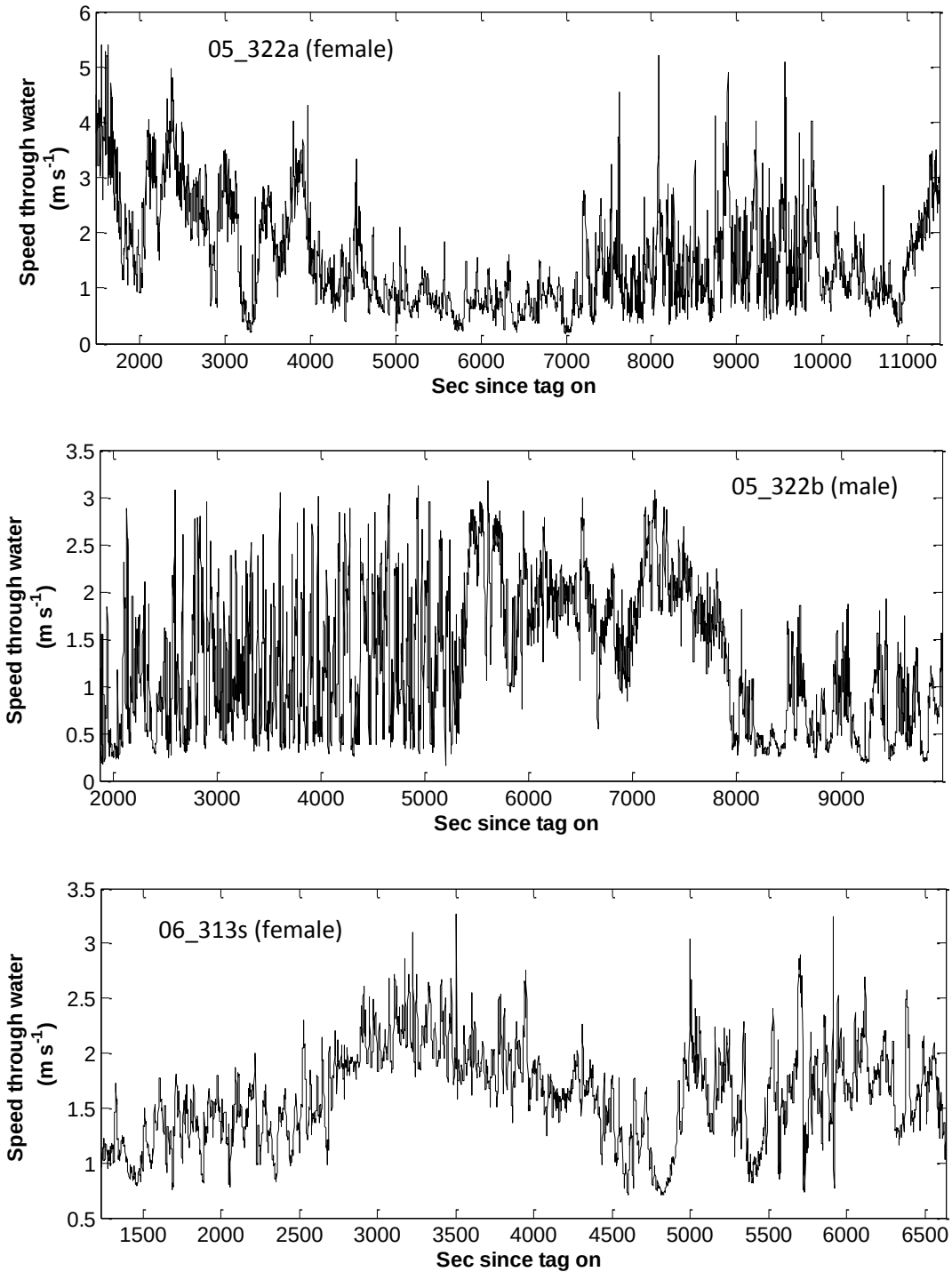

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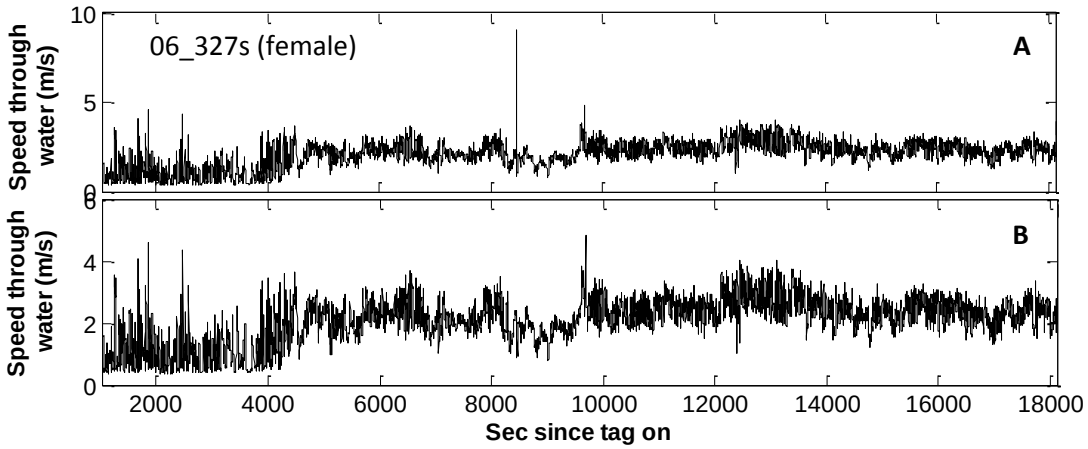

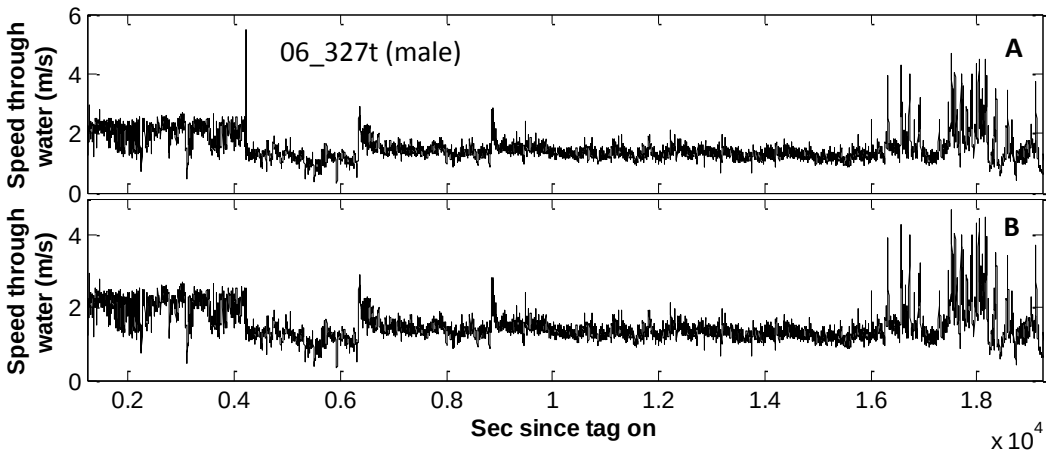

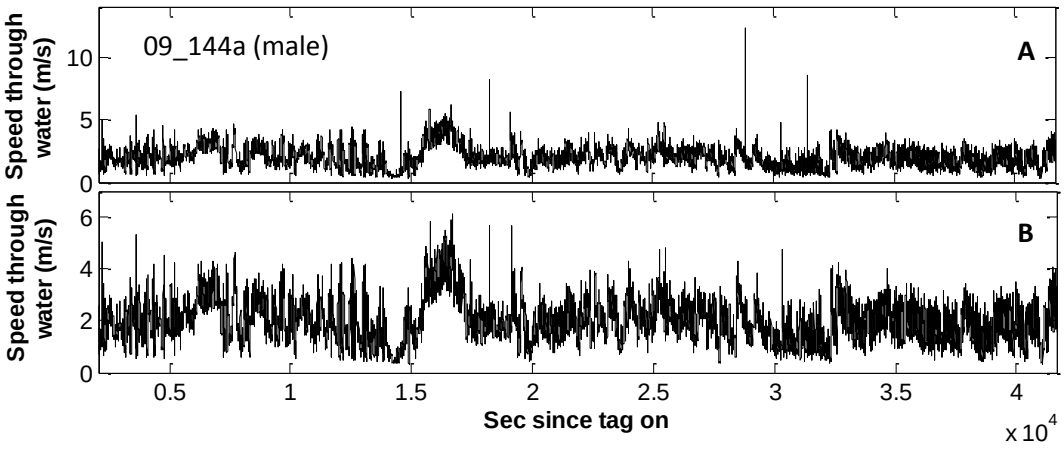

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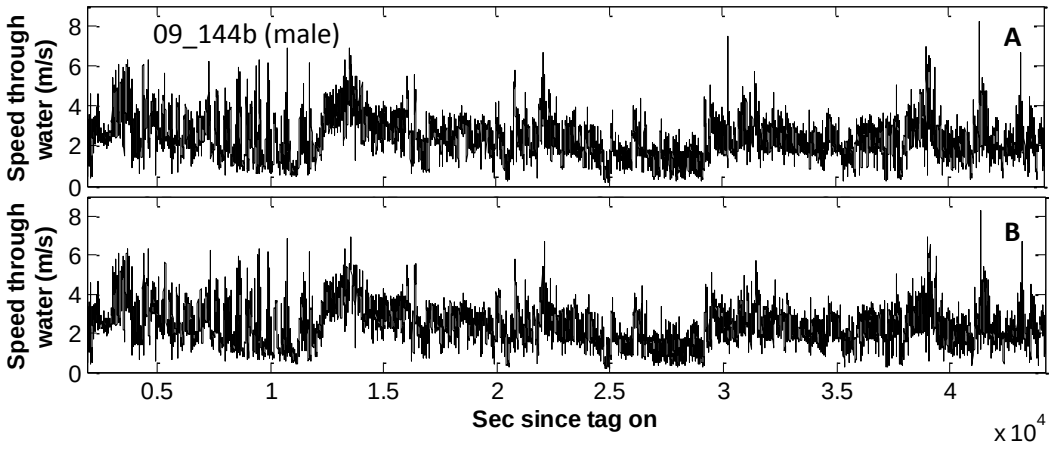
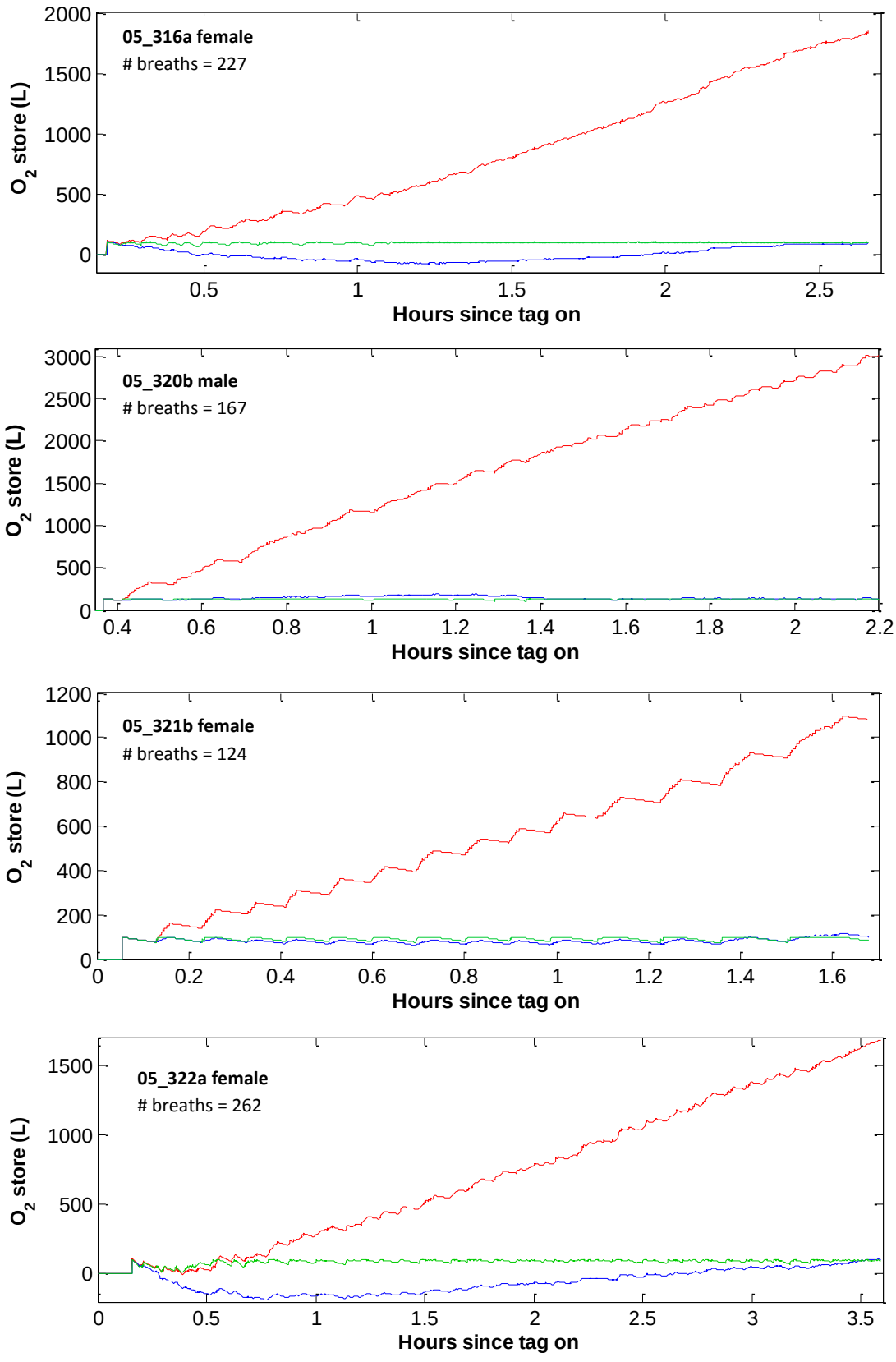


Appendix 2.2. Time-series plots of the O_2 store over the entire tag records per individual killer whale estimated through speed applied to the O_2 model using a fixed O_2 uptake (red), optimized fixed O_2 uptake (blue), and a fluctuating O_2 uptake according to a set O_2 uptake curve (green).



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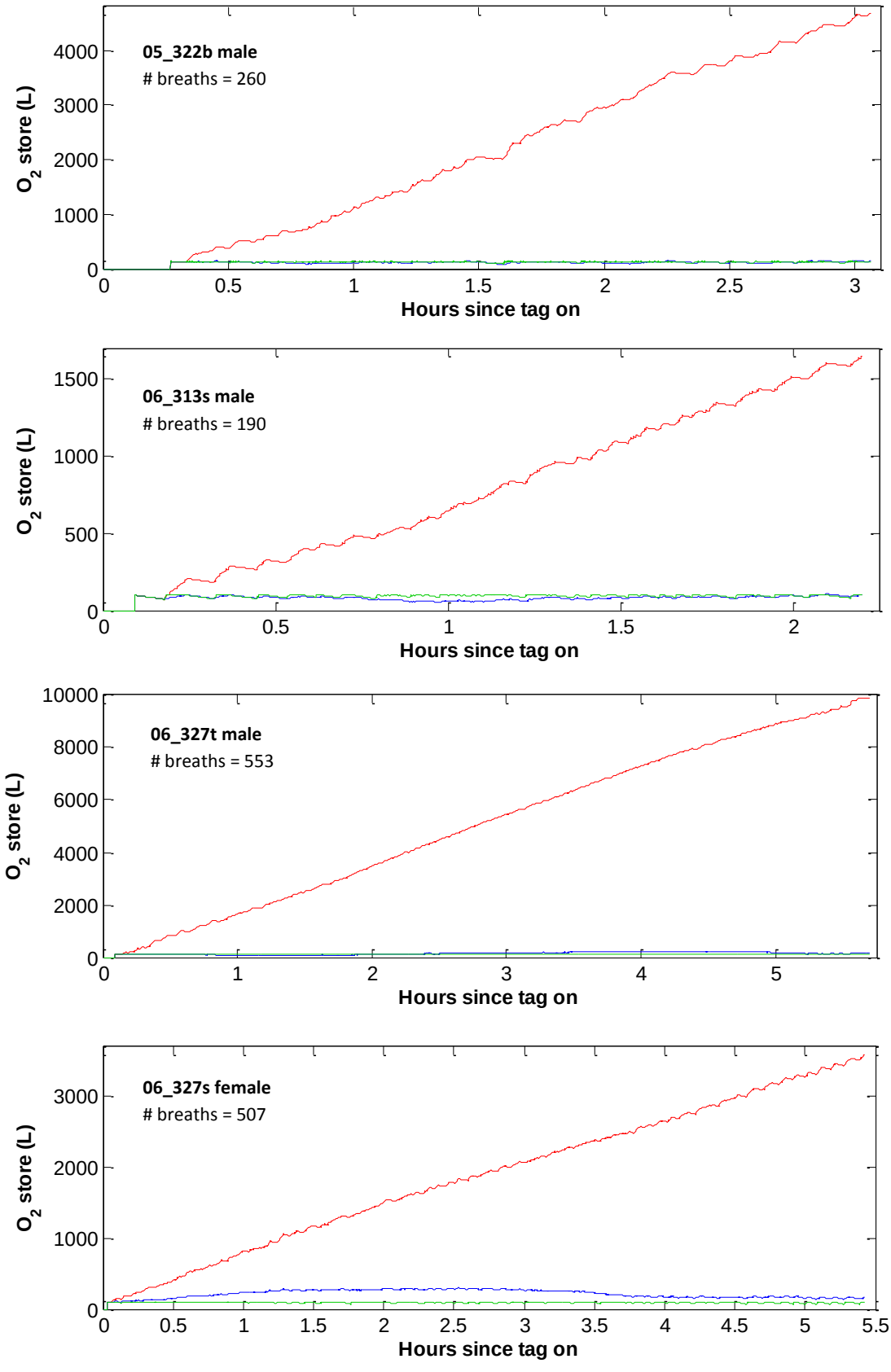

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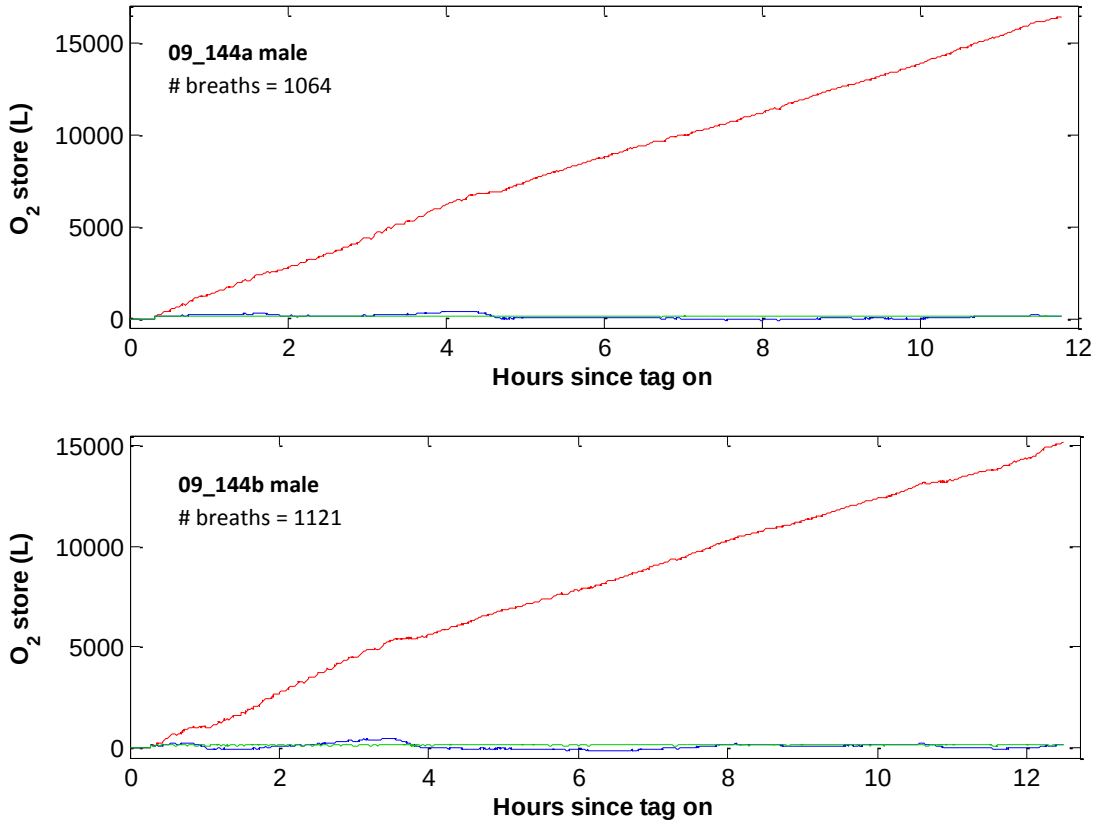
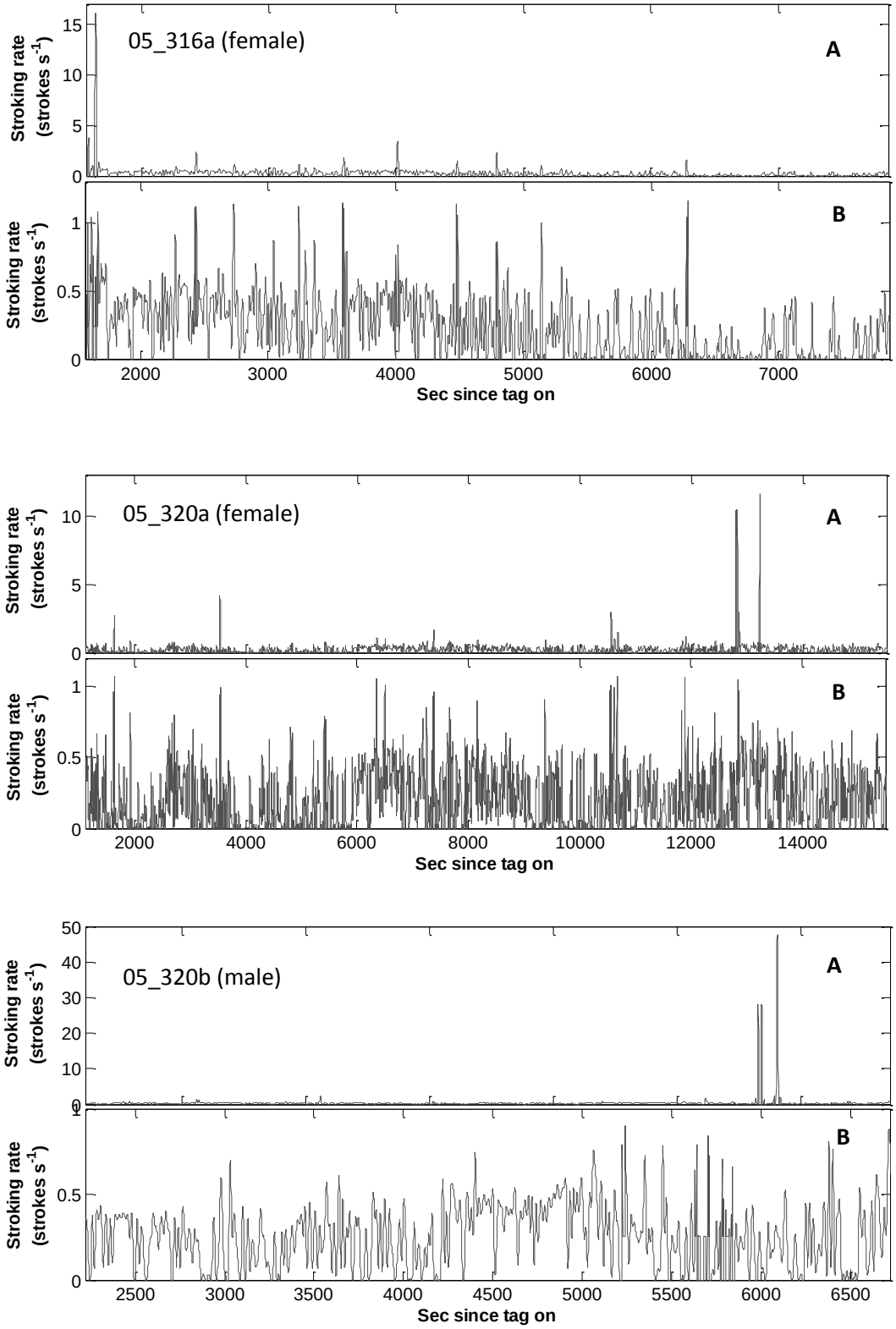


Appendix 3.1. Time series plots of the stroking rate measurements per individual killer whale before (A) and after (B) removing outliers.



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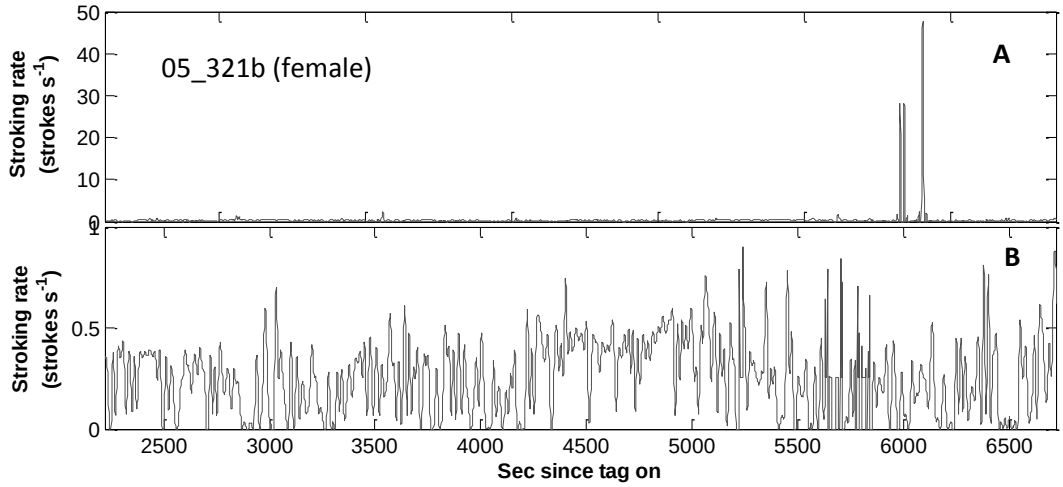

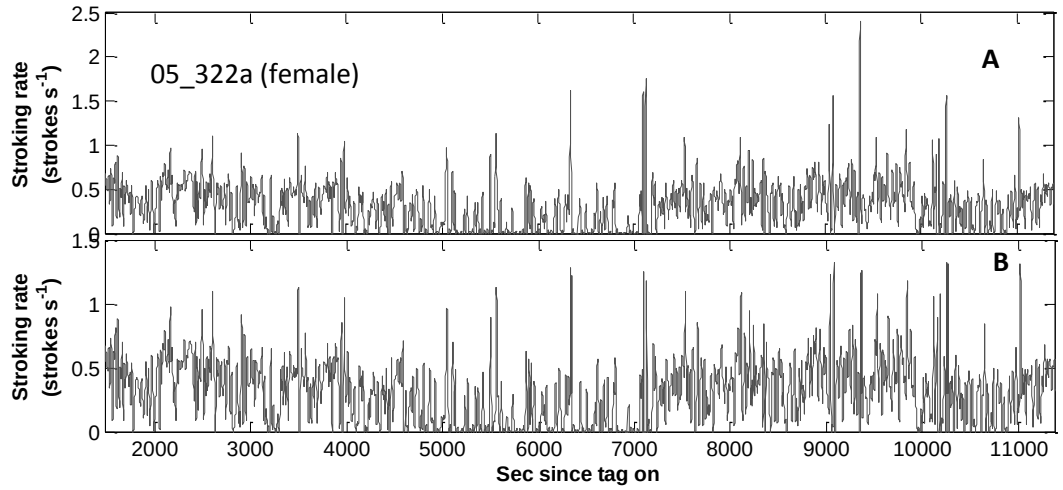

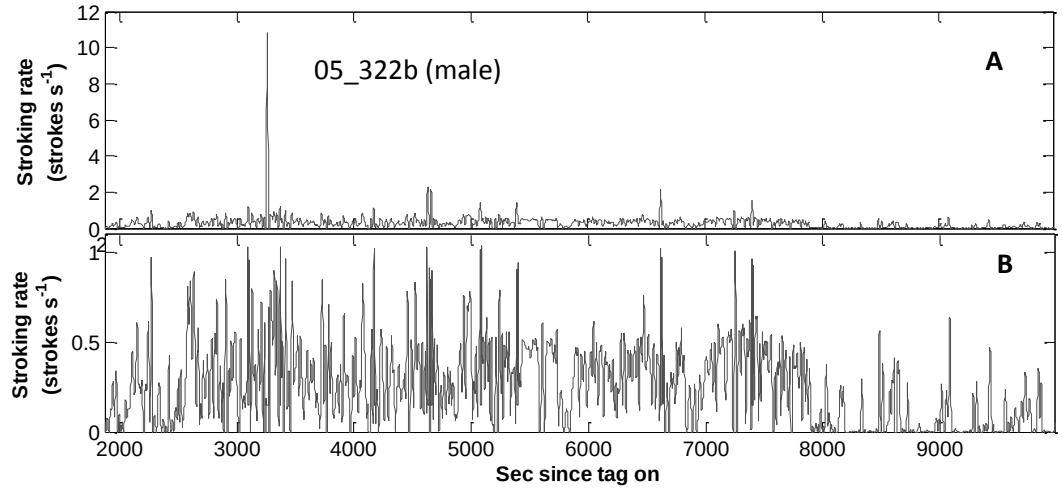

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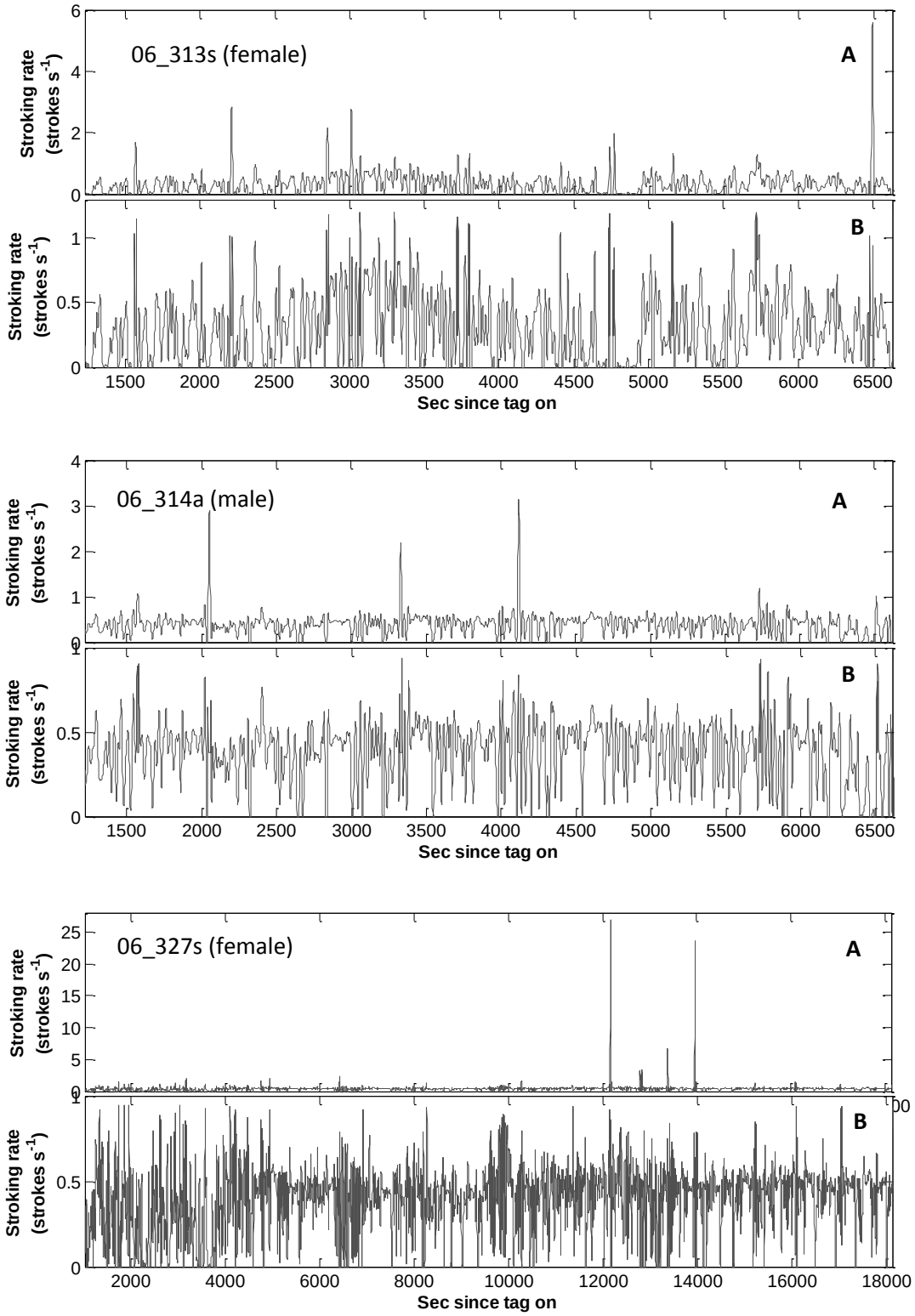

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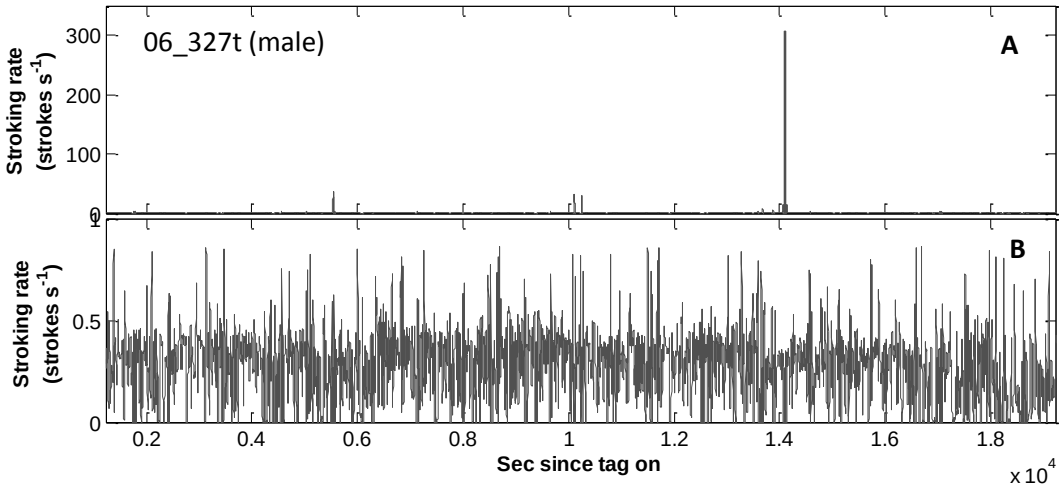
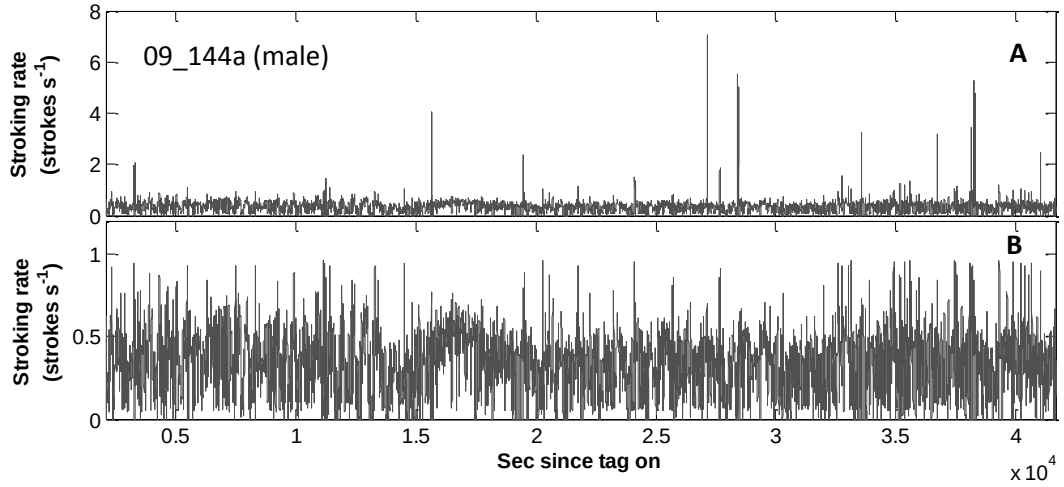
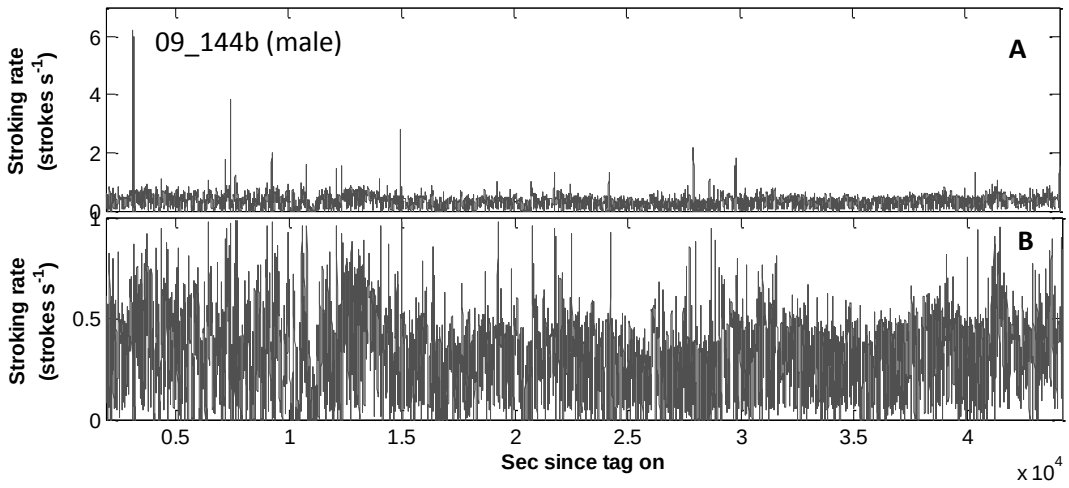


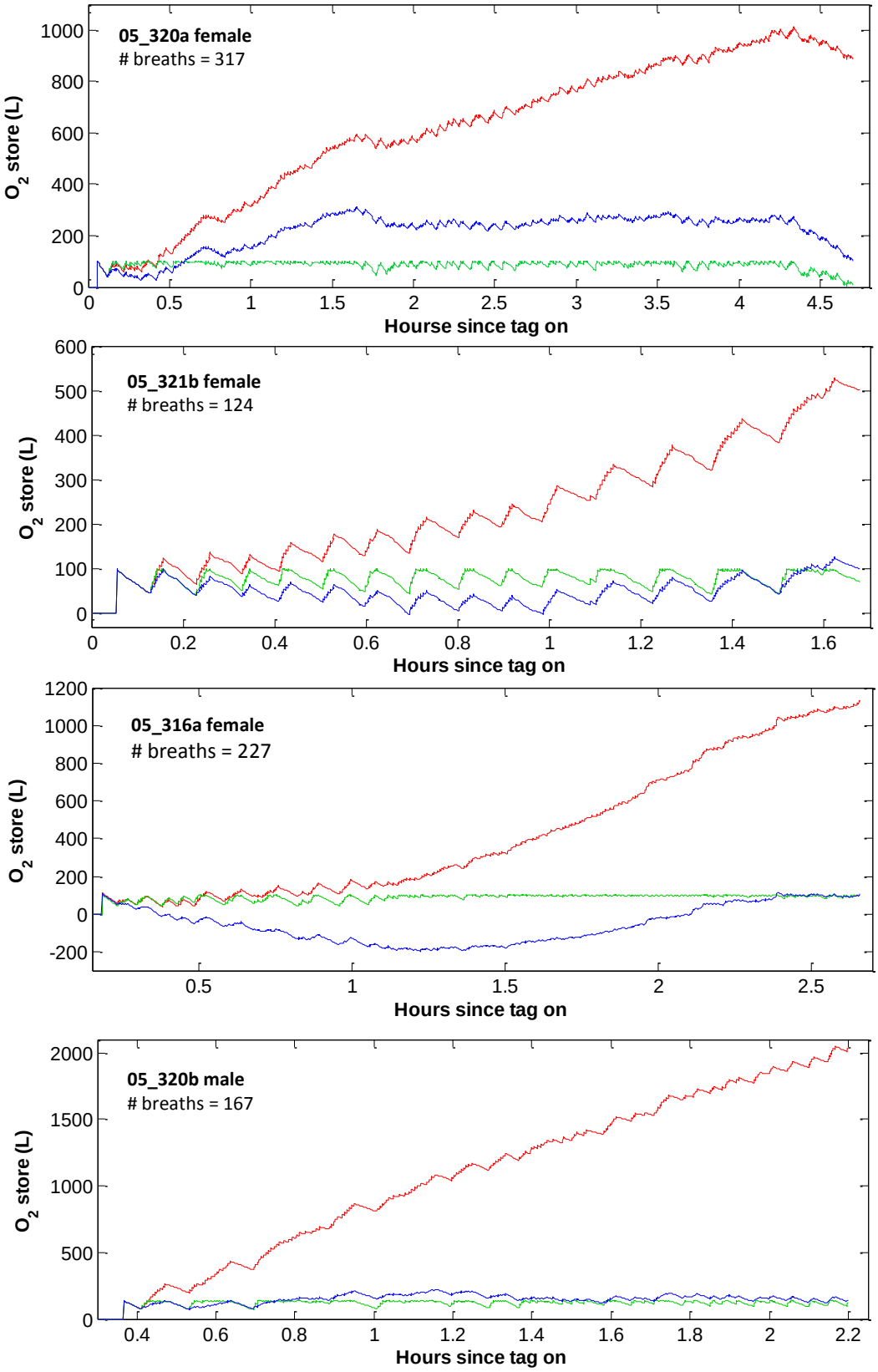




Appendix 3.2. Statistics on 1s outliers detected and replaced in stroking rate measurements per individual killer whale. Statistics are related to tag record periods included in analyses

Whale ID	Gender	# 1s interval outliers	% of tag record
05_316a	Female	31	0.49
05_320a	Female	31	0.22
05_320b	Male	37	0.82
05_321b	Female	17	0.47
05_322a	Female	39	0.39
05_322b	Male	55	0.68
06_313s	Female	40	0.74
06_314a	Male	32	0.59
06_327s	Female	85	0.50
06_327t	Male	103	0.57
09_144a	Male	332	0.84
09_144b	Male	181	0.43

Appendix 3.3. Time-series plots of the O_2 store over the entire tag records per individual killer whale estimated through stroking rate applied to the O_2 model using a fixed O_2 uptake (red), optimized fixed O_2 uptake (blue), and a fluctuating O_2 uptake according to a set O_2 uptake curve (green).



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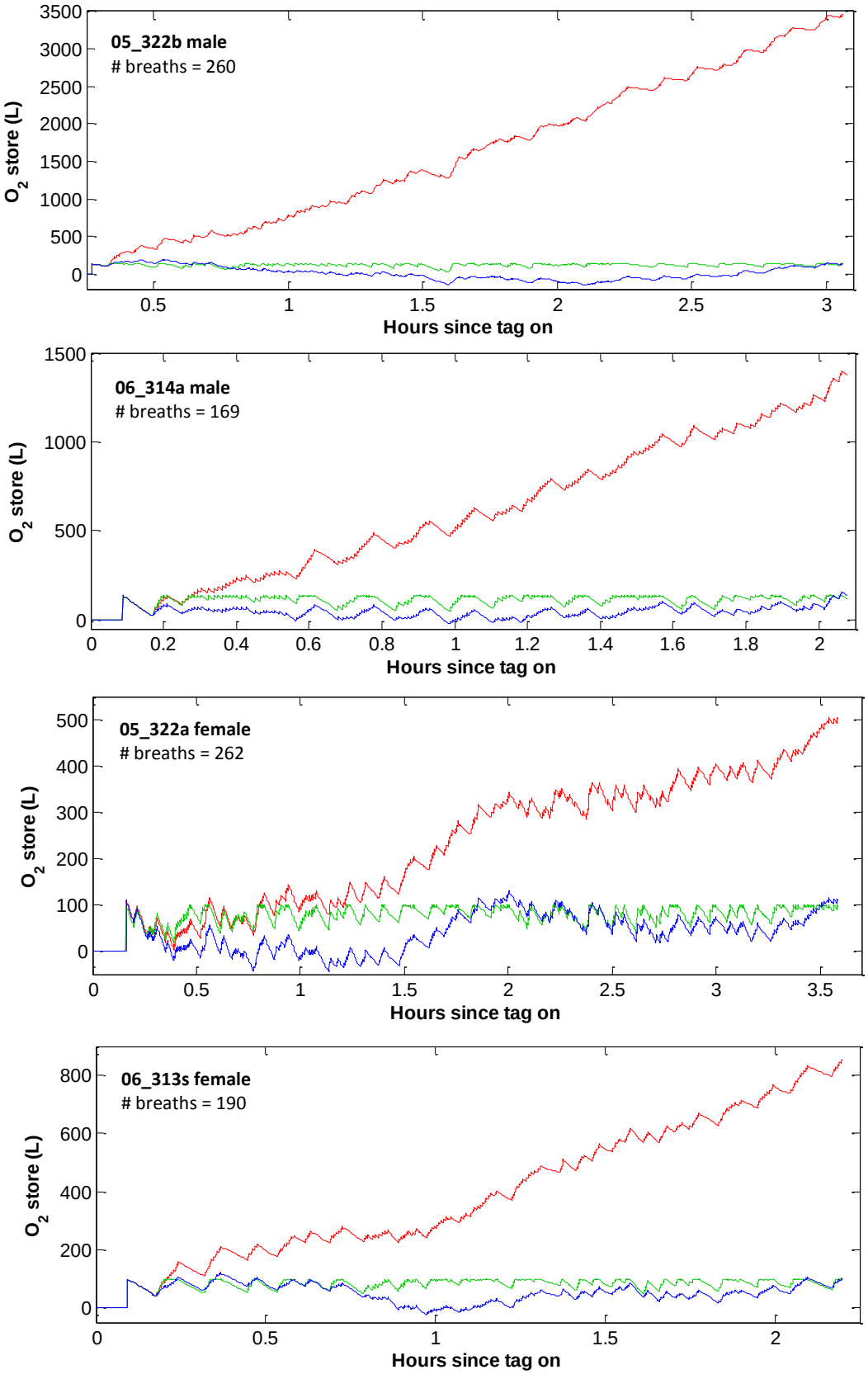

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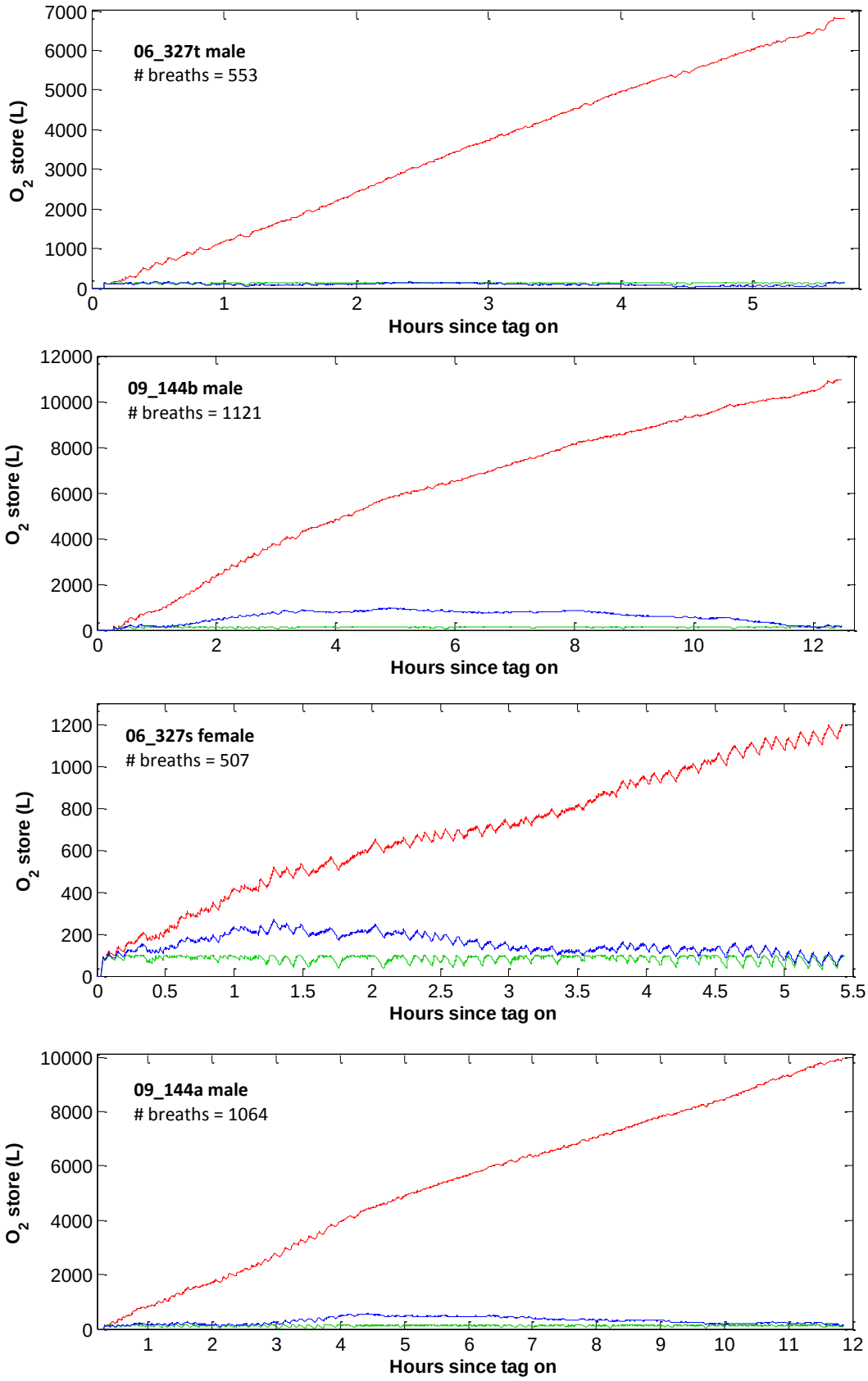
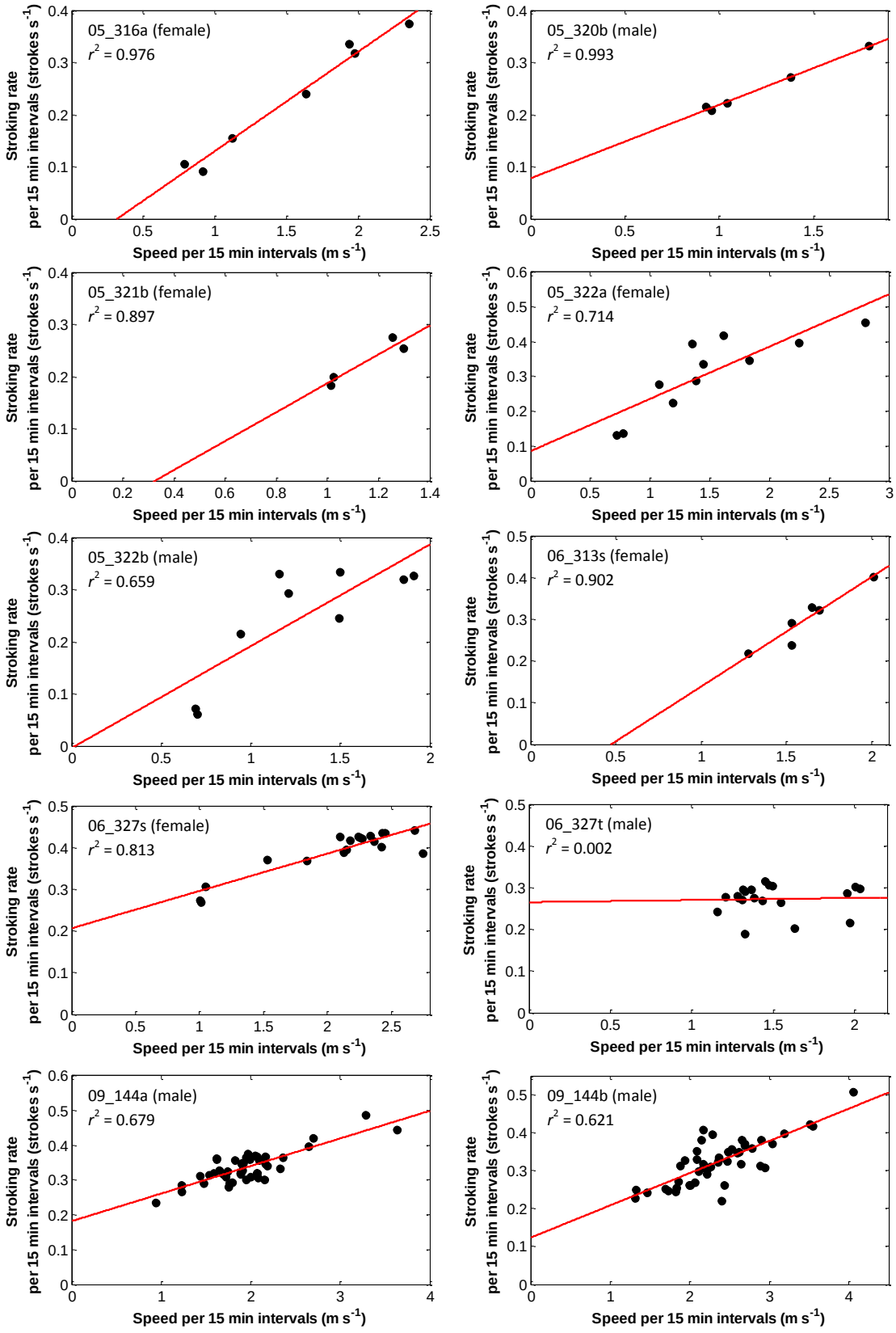


Appendix 4.1. Strength of the correlation (r^2) between estimated E_{O2} and level of activity over 15 min intervals, according to all O_2 model variants and both activity metrics, per sex category and per individual killer whale. Correlation strength values showing at least a 0.01 increase with the value by using the other metric for the same model variant are coloured.

	O_2 model using fixed E_{O2}		O_2 model using optimized E_{O2}		O_2 model including O_2 uptake curve		O_2 model including optimized O_2 uptake curve	
	Stroking r^2	Speed r^2	Stroking r^2	Speed r^2	Stroking r^2	Speed r^2	Stroking r^2	Speed r^2
All females	-15.541	-26.313	-11.262	-4.371	0.979	0.899	0.979	0.903
All males	-0.751	-27.400	-0.362	-3.675	0.950	0.954	0.954	(0.953)
Females								
05_316a	-12.675	-46.174	-8.927	-7.440	0.976	0.990	0.988	0.996
05_320a	-8.730	-	-6.814	-	0.903	-	(0.902)	-
05_321b	-44.398	-175.528	-31.063	-4.696	0.975	0.952	(0.974)	0.983
05_322a	-0.085	-19.391	0.068	-5.139	0.982	0.997	(0.981)	(0.997)
06_313s	-0.515	-2.773	-0.260	0.201	0.959	0.976	(0.958)	0.982
06_327s	-1.210	-131.023	-0.935	-31.440	0.942	0.981	0.948	0.983
Males								
05_320b	-3.872	-126.019	-2.177	-5.325	0.955	0.784	(0.954)	0.783
05_322b	-1.374	-14.945	-0.250	0.531	0.970	0.908	0.970	(0.907)
06_314a	0.584	-	0.565	-	0.886	-	0.926	-
06_327t	0.452	-82.653	0.600	-3.816	0.956	0.912	0.968	(0.911)
09_144a	-0.149	-53.733	0.012	-12.276	0.889	0.938	0.916	0.971
09_144b	-0.007	-19.924	0.086	-8.803	0.931	0.907	0.935	0.941

Appendix 4.2. Correlation between stroking rate and speed per 15 min intervals per individual killer whales, with lines showing simple linear regression models.



Appendix 4.3. Correlation between O_2 use (including.BMR) estimated by stroking and speed^3 per 15 min intervals per individual killer whales, with lines showing simple linear regression models.